Workshop

Liver-Gut-Microbiome Interactions

January 25 – 26, 2018
Radisson Blu Hotel
Hamburg, Germany

Abstracts
Poster Abstracts

FALK FOUNDATION e.V.

Leinenweberstr. 5
79108 Freiburg
Germany

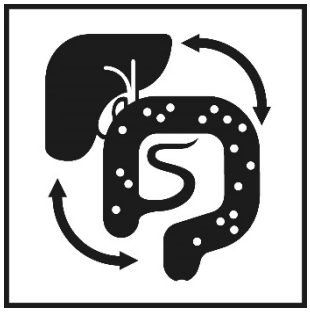
www.falkfoundation.org

© 2018 Falk Foundation e.V.
All rights reserved.

Abstracts of Invited Lectures
Poster Abstracts

Workshop

LIVER-GUT-MICROBIOME INTERACTIONS



Hamburg, Germany
January 25 – 26, 2018

Scientific Organization:
A.W. Lohse, Hamburg (Germany)

Scientific Co-Organization:
N. Gagliani, Hamburg (Germany)
S. Huber, Hamburg (Germany)
C. Schramm, Hamburg (Germany)

CONTENTS

Welcome and opening remarks
A.W. Lohse, Hamburg

page

Session I

Intestinal inflammation

Chair:
S. Huber, Hamburg
H. Tilg, Innsbruck

Intestinal inflammation and its relevance for the liver
M.F. Neurath, Erlangen 15 – 16

DC as central players of mucosal immunology
E.J. Villablanca, Stockholm 17 – 18

Mucosal barrier function in health and disease (no abstract)
J. Wehkamp, Tübingen

State-of-the-Art Lecture:
IL-10-producing Tr1 regulatory cells: From the bench to the bedside
M.G. Roncarolo, R. Bacchetta, S. Gregori, Stanford, Milan 19

Session II

Microbiome

Chair:
J.G. Bode, Düsseldorf
A. Stallmach, Jena

Diet and microbiota: Potential for therapeutic interventions
S.C. Bischoff, Stuttgart 23

How genes shape our microbiota (no abstract)
A. Franke, Kiel

Neonatal imprinting of tolerogenic properties in gut-draining lymph nodes by microbiota
J. Huehn, Braunschweig 24

Regulation of mucosal immunity and barrier function by the intestinal microbiota
T. Strowig, Braunschweig 25

Lipid metabolism, microbiota and inflammation
J. Heeren, Hamburg 26

Oral poster presentation
A novel mouse model to study IL-6/gp130 signaling and acute-phase proteins in the gut-liver axis
N. Schumacher, M. Müller, K. Lücke, T. Wunderlich, H. Lotter, N. Gagliani, H.-W. Mittrücker, A. Rehman, S. Rose-John, D. Schmidt-Arras, Kiel, Hamburg, Cologne 27

Oral poster presentation
Inhibition of gut microbiota can be a therapeutic target to reduce liver fibrosis
F. El Zahraa Ammar Mohamed, S. Dooley, N.A. Davies, S. Hammad, A. Habtesion, R. Jalan, Mannheim, Minia, Qena, London 28

Oral poster presentation
IgA antibodies against filamentous-actin are frequently detected in patients with cirrhosis and indicate a progressive disease course
T. Tornai, B. Balogh, N. Sipeki, Z. Vitalis, I. Tornai, P. Antal-Szalmas, T. Dinya, G.L. Norman, T. Bruns, M. Papp, Debrecen, San Diego, Jena 29 – 30

Oral poster presentation
L-selectin (CD62L) is increased in patients with ulcerative colitis and drives the progression of non-alcoholic steatohepatitis (NASH) in mouse and men
H.K. Drescher, A. Schippers, H. Sahin, C. Trautwein, D.C. Kroy, Aachen 31

Session III

Metabolism and inflammation

Chair:
N. Gagliani, Hamburg
M.F. Neurath, Erlangen

Fatty liver disease: Role model of immunometabolic diseases?
F. Tacke, Aachen 35

Influence of diurnal factors on the microbiome (no abstract)
E. Elinav, Rehovot

State-of-the-Art Lecture:
How to maintain liver immune tolerance
P.A. Knolle, Munich 36

Session IV

Liver inflammation

Chair:
R. Thimme, Freiburg
C. Trautwein, Aachen

Type I IFN in liver fibrosis; an indisputable harassing factor
Z. Abdullah, Bonn 39

Adaptive immune cells and liver inflammation
M. Iannacone, Milan 40

Consequences of liver inflammation (no abstract)
N.N.

Therapeutic targets in the liver gut axis – Evolving concepts
(no abstract)
D.H. Adams, Birmingham

Closing remarks

Opening of the Annual Meeting of the GASL

List of Chairpersons, Speakers and Scientific Organizers 41 – 42

Poster Abstracts

1. Morpho-functional changes of lymphoid thymus cells on the background of prolonged use of proton-pump inhibitors
   A.V. Antonenko, S.V. Pylypenko, O.H. Korotkyi, T.V. Beregova, L.I. Ostapchenko (Kiev, Poltava, UA)

2. NAFLD and intestinal microbiota: Clinical parallels
   I. Bakulin, M.P. Abatsieva, L.N. Belousova (St. Petersburg, RU)

3. The influence of an altered microbiota and PSC on colitis severity
   S. Bohmann, T. Bedke, C. Haueis, N. Gagliani, S. Huber (Hamburg, DE)

4. Mechanisms how gut-derived bacterial toxin (LPS) affects liver cells
   K. Breitkopf-Heinlein, H. Gaitantzi, C. Cai, M.P. Ebert (Mannheim, DE)

5. L-selectin (CD62L) is increased in patients with ulcerative colitis and drives the progression of non-alcoholic steatohepatitis (NASH) in mouse and men
   H. Drescher, A. Schippers, H. Sahin, C. Trautwein, D. Kroy (Aachen, DE)

6. Non-invasive diagnostic of liver fibrosis in patients with type 2 diabetes with complications in daily practice
   K. Dvorak, Y. Barbora, A. Hovorkova, J. Vejrychova (Liberec, CZ)

7. Analysis of murine and human histone deacetylase 7 expression in fibrosis and its subcellular localization during inflammation
   K. Freese, J. Sommer, C. Hellerbrand (Erlangen, DE)

8. A novel MAPK14-ATF2-microRNA axis contributes to hepatocellular carcinoma progression and sorafenib resistance
   V. Fritz, L. Malek, C. Hellerbrand, A.K. Bosserhoff, P. Dietrich (Erlangen, DE)

9. Human 3-dimensional intestinal and liver organoids: In vitro model system to study the gut-liver axis
   H. Gaitantzi, C. Cai, N. Rindtorff, J. Betge, M.P. Ebert, K. Breitkopf-Heinlein (Mannheim, Heidelberg, DE)

10. Expression and function of neuroblastoma RAS viral oncogene homolog in hepatocellular carcinoma
    A. Gaza, C. Hellerbrand, A.K. Bosserhoff, P. Dietrich (Erlangen, DE)

11. The role of IL-22 and IL-22BP in liver metastasis
    A. Giannou, J. Kempski, L. Garcia Perez, A.M. Shiri, L. Brockmann, D. Zazara, J. Lücke, L. Zhao, T. Bedke, T. Agalioti, N. Gagliani, S. Huber (Hamburg, DE)

12. Reduction of carcinogenesis-associated pathways by pharmacological inhibition of the cannabinoid receptor 1 in the murine Abcb4$^{-/-}$ mouse model
    N.L. Helmrich, Y. Churin, A. Tschuschner, M. Roderfeld, E. Roeb (Gießen, DE)

13. Dietary cholesterol promotes transition from hepatic steatosis to steatohepatitis/ NASH particularly in combination with a PUFA-rich Western-type diet
J. Henkel, M. Kuna, D. Coleman, F. Gellert, J.P. Castro, G. Püschel (Nuthetal, DE)

14. Identification of the binding peptide sequence of cytokeratin 18 to the large hepatitis B surface protein
A. Huhn, Y. Churin, D. Glebe, T. Eckert, D. Schröder, M. Roderfeld, E. Roeb (Gießen, Idstein, DE)

15. Hepatic CD36-dependent accumulation of lipids in HBs transgenic mice
K. Irungbam, Y. Churin, A. Tschuschner, D. Leder, M. Roderfeld, E. Roeb (Gießen, DE)

16. Beneficial effects of vitamin D treatment in an obese mouse model of non-alcoholic steatohepatitis
D. Jahn, D. Dorbath, S. Kircher, H.M. Hermanns, A. Geier (Würzburg, DE)

17. Pilot single-center study of measurement of liver stiffness of patients with ulcerative colitis and Crohn's disease on long-term therapy with thiopurines
G. Karampekos, G. Fillipidis, N. Viazis, G.J. Mantzaris (Athens, GR)

18. Prospective, longitudinal cohort study of the pathologic increase in liver stiffness: Early diagnosis of liver disease in cystic fibrosis
V. Klotter, C. Gunchick, E. Siemers, H. Hudel, M. Roderfeld, E. Roeb (Gießen, DE)

19. Effects of combined low-dose spironolactone plus vitamin E vs. vitamin E monotherapy on insulin resistance, non-invasive indices of steatosis and fibrosis, and adipokine levels in non-alcoholic fatty liver disease: A randomized controlled trial
J. Kountouras, S.A. Polyzos, C.S. Mantzoros, V. Polymerou, P. Katsinelos, M. Doulberis (Thessaloniki, Athens, GR; Boston, US; Solothurn, CH)

20. Serum sterol levels indicate distorted cholesterol homeostasis in cirrhotic patients with primary biliary cholangitis
M. Krawczyk, E. Wunsch, D. Lütjohann, F. Lammert, P. Milkiewicz (Homburg, Bonn, DE; Szczecin, Warsaw, PL)

21. The intestinal microbiota of patients with PSC are different from healthy controls and patients with ulcerative colitis across geographical regions
T. Liwinski, F. Heinsen-Groth, M. Rühlemann, R. Zenouzi, M. Kummen, J.R. Hov, T.H. Karlsen, C. Bang, A.W. Lohse, A. Franke, C. Schramm (Hamburg, Kiel, DE; Oslo, NO)

22. Combined effects of curcumin and xanthohumol in *in vitro* models of hepatic steatosis and fibrosis
A. Mahli, T. Seitz, A. Chiet, C. Hellerbrand (Erlangen, DE)

23. Inhibition of gut microbiota can be a therapeutic target to reduce liver fibrosis
F. Mohamed, S. Dooley, N. Davies, S. Hammad, A. Habtesion, R. Jalan (Mannheim, DE; London, GB; Qena, Minia, EG)

24. *Schistosoma mansoni* activates protooncogene c-Jun in hamster model
S. Padem, C.G. Grevelding, T. Quack, Y. Churin, A. Tschuschner, M. Roderfeld, E. Roeb (Gießen, DE)

25. Microbiota-dependent effects of IL-22
P. Pelczar, M. Said, M. Böttcher, S. Huber (Hamburg, DE)

26. Oral probiotic administration influences pro- and anti-inflammatory cytokines in NAFLD
O.M. Plehutsa, I.R. Sydorchuk, A.R. Sydorchuk, L.P. Sydorchuk, O. Khomko, I.I. Sydorchuk, R.I. Sydorchuk, O.B. Rusak (Chernivtsi, UA; Frankfurt, DE)

27. 12/15-Lipoxygenase-deficient mice show exacerbated experimental colitis and alterations in epithelial proliferation
M. Ragab, A. Sünderhauf, K. von Medem, F. Bär, R. Pagel, A. Künstner, C. Sadik, S. Derer, C. Sina (Lübeck, DE)

28. IL-13 knockout reduces the activation of ER stress associated pathways in Abcb4$^{-/-}$ mice
M. Roderfeld, L. Hahn, A. Tschuschner, E. Roeb (Gießen, DE)

29. Optimisation of liver cell quantification in 3D scaffolds
M. Ruoß, C. Grom-Baumgarten, L. Olde Damink, S. Lee, A.K. Nüssler (Tübingen, Herzogenrath, Munich, DE)

30. Effects of short-term dietary changes on immune system and beyond
N. Schaltenberg, L. Fromann, P. Scognamilio, T. Agalioti, A. Fischer, P. Pelzcar, A. Worthmann, R. Wahib, M. Heine, L. Scheja, S. Huber, J. Heeren, N. Gagliani (Hamburg, DE; Solna, SE)

31. Knockout of endocannabinoid receptor 1 reduces hepatic steatosis in a mouse model of chronic hepatitis B
F. Schneider, K. Irungbam, Y. Churin, M. Roderfeld, E. Roeb (Gießen, DE)

32. A novel mouse model to study IL-6/gp130 signaling and acute-phase proteins in the gut-liver axis
N. Schumacher, M. Müller, K. Lücke, T. Wunderlich, H. Lotter, N. Gagliani, H.-W. Mittrücker, A. Rehman, S. Rose-John, D. Schmidt-Arras (Kiel, Hamburg, Cologne, DE)

33. Xanthohumol, a prenylated chalcone derived from hops, inhibits hepatic metastasis of tumor cells
T. Seitz, P. Dietrich, C. Hackl, A. Mahli, S. Lang, A.K. Bosserhoff, C. Hellerbrand (Erlangen, Regensburg, Freiburg, DE)

34. Effect of diet, physical activity, sleep and defecation on quality of life
K.A. Shemerovskii (St. Petersburg, RU)

35. Epithelial cell-derived complement component 3 is involved in intestinal immune responses during chronic colitis
K. Skibbe, A. Sünderhauf, S. Preisker, K. Ebbert, A. Verschoor, C.M. Karsten, C. Kemper, M. Basic, A. Bleich, C. Sina, S. Derer
(Lübeck, Hannover, DE; Bethesda, US; London, GB)

36. Analysis of the effects of ethanol on expression of factors promoting hepatic metastasis in hepatoma and melanoma cells
J. Sommer, K. Freese, C. Hellerbrand (Erlangen, DE)

37. Analysis of the interaction between inflammatory bowel disease and liver pathology in a murine model
N. Steffens, T. Bedke, S. Huber (Hamburg, DE)

38. Saccharin supplementation modulates the intestinal microbiome and is protective in early stages of experimental colitis
A. Sünderhauf, A. Künstner, R. Pagel, A. Wagner, S. Ibrahim, S. Derer, C. Sina (Lübeck, DE)

39. Linking mucus depletion and microbiome alterations in UC to mucosal energy supply
A. Sünderhauf, F. Bär, M. Hirose, A. Künstner, R. Pagel, S. Ibrahim, S. Derer, C. Sina (Lübeck, DE)

40. Possible common genetic background for metabolic and immune disorders in hepatic steatosis, obesity and hypertension
A.R. Sydorchuk, L.P. Sydorchuk, O.M. Plehutsa, Y. Yarynych, R.I. Sydorchuk, I.I. Sydorchuk (Chernivtsi, UA)

41. ACE (I/D) and AGTR1 (A1166C) genes single nucleotide polymorphisms, non-alcoholic fatty liver disease and intestinal dysbiosis
L.P. Sydorchuk, R.I. Sydorchuk, O.V. Kushnir, O.M. Iftoda, A.R. Sydorchuk, I.I. Sydorchuk, O.M. Plehutsa, O. Khomko (Chernivtsi, UA)

42. Intestinal barrier suffer major impact in acute enteral dysfunction syndrome due to gut microflora, antiendotoxin core antibodies and nitric oxide associated vicious circle
R.I. Sydorchuk, L.P. Sydorchuk, O. Kolomojets, O.M. Plehutsa, I.I. Sydorchuk, O. Khomko, A.R. Sydorchuk (Chernivtsi, UA)

43. IgA antibodies against filamentous-actin are frequently detected in patients with cirrhosis and indicate a progressive disease course
T. Tornai, B. Balogh, N. Sipeki, Z. Vitalis, I. Tornai, P. Antal-Szalmas, T. Dinya, G.L. Norman, T. Bruns, M. Papp (Debrecen, HU; San Diego, US; Jena, DE)

44. Functional polymorphisms of innate immunity pattern recognition receptors do not constitute the risk of bacterial infections other than spontaneous bacterial peritonitis and also not the progressive disease course in patients with cirrhosis
T. Tornai, T. Dinya, B. Balogh, Z. Vitalis, I. Tornai, D. Tornai, P. Antal-Szalmas, H. Andirkovics, A. Bors, A. Tordai, M. Papp (Debrecen, Budapest, HU)

45. Single microRNA modulation in hepatocytes: A promising treatment for liver fibrosis
H.-C. Tsay, Q. Yuan, A. Balakrishnan, M. Kaiser, M. Farid, S. Möbus, A. Kispert, M.P. Manns, M. Ott, A.D. Sharma (Hannover, DE; Cairo, EG)

46. Nrf2 and c-Met on hepatocytes is protective during the development of non-alcoholic steatohepatitis (NASH)
L. Van den Burg, H. Drescher, S. Erschfeld, C. Trautwein, D. Kroy (Aachen, DE)

47. German HCV(1b)-Anti-D cohort – Long-term history and treatment results
M. Wiese; East German HCV Study Group (Leipzig, DE)

48. Characterization of primary sclerosing cholangitis (PSC)-associated inflammatory bowel disease
A.F. Wittek, B. Steglich, S. Huber (Hamburg, DE)

Session I

Intestinal inflammation

Intestinal inflammation and its relevance for the liver

Markus F. Neurath
Department of Medicine 1, Kussmaul Campus for Medical Research, University of Erlangen-Nuremberg, Germany

Inflammatory bowel diseases (Crohn´s disease, ulcerative colitis) are chronic inflammatory disorders of the gastrointestinal tract. Studies on disease pathogenesis have suggested that IBD develop by uncontrolled activation of the mucosal immune system in a genetically susceptible host. This activation appears to be triggered by antigens from the commensal microflora. Predisposing factors consist of genetic factors and environmental factors including smoking, NSAID use, infections and antibiotic therapy. Such factors appear to cause an alteration of the mucosal barrier function thereby facilitating the translocation of bacteria from the commensal microflora into the bowel wall. Subsequently, an uncontrolled activation of the mucosal immune system occurs leading to intestinal inflammation and potential side effects and complications such as stenosis and cancer.

Recent studies have unequivocally demonstrated that both innate and adaptive cellular immunity are altered in IBD [1–4]. This includes changes in antimicrobial host defense and immune responses by intestinal epithelial cells, Paneth cells, granulocytes, macrophages and dendritic cells as well as marked alterations of lymphocyte responses including B and T cell responses [5, 6]. These changes are not only relevant for the pathogenesis but also for the therapy of IBD.

This presentation will give a brief overview about the observed changes in innate and adaptive immune responses in IBD. The implications of recent findings on the design on novel therapeutic approaches for IBD as well as for individualized therapy in IBD will be discussed. Moreover, we will discuss the potential implications of bacteria-driven mucosal inflammation in IBD in the context of liver inflammation and steatosis. Finally, we will review the potential crosstalk between the immune system of the gut and the liver in patients with primary sclerosing cholangitis.

References:

1. Neurath MF. Cytokines in inflammatory bowel disease. Nat Rev Immunol. 2014;14:329–42.

2. Strober W. The regulation of mucosal immune system. J Allergy Clin Immunol. 1982;70:225–30.

3. Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. J Clin Invest. 2007;117:514–21.

4. Danese S, Fiocchi C. Ulcerative colitis. N Engl J Med. 2011;365:1713–25.

5. Wehkamp J, Salzman NH, Porter E et al. Reduced Paneth cell alpha-defensins in ileal Crohn's disease. Proc Natl Acad Sci USA. 2005;102:18129–34.

6. Gerlach K, Hwang Y, Nikolaev A et al. TH9 cells that express the transcription factor PU.1 drive T cell-mediated colitis via IL-9 receptor signaling in intestinal epithelial cells. Nat Immunol. 2014;15:676–86.

DC as central players of mucosal immunology

Eduardo J. Villablanca
Immunology and Allergy Unit, Department of Medicine (Solna), Karolinska Institute and University Hospital, Stockholm, Sweden

Complex interactions between the intestinal epithelial barrier, microbiota and host immune system are tightly regulated to maintain intestinal homeostasis. Such interactions must maintain the balance between effector immunity against pathogens and unresponsiveness against commensal microorganisms; breakdown in this balance might precipitate the onset of intestinal disorders, such as inflammatory bowel disease (IBD). Dendritic cells (DC), due to their abilities to induce inflammatory responses and tolerance in response to pathogenic and commensal bacteria, are key in maintaining intestinal homeostasis. Our laboratory and others have identified that retinoic acid (RA), metabolized from the dietary Vitamin A, is essential to imprint DC with tolerogenic properties as well as the ability to induce gut-homing tropism on lymphocytes.

Here I will discuss the immunological influence of some dietary compounds and their metabolites acting on DC imprinting. In particular, I will discuss the role of RA in regulating gut DC function and homing properties. I will also discuss some unpublished data from our own lab showing the role of cholesterol sensing on intestinal DC-mediated immunity.

References:

1. Belkaid Y, Artis D. Immunity at the barriers. Eur J Immunol. 2013;43:3096–7.
2. Belkaid Y, Harrison OJ. Homeostatic Immunity and the Microbiota. Immunity. 2017;46:562–76.
3. de Souza HS, Fiocchi C. Immunopathogenesis of IBD: current state of the art. Nat Rev Gastroenterol Hepatol. 2016;13:13–27.
4. Mowat AM, Agace WW. Regional specialization within the intestinal immune system. Nat Rev Immunol. 2014;14:667–85.
5. Cassani B, Villablanca EJ, De Calisto J, Wang S, Mora JR. Vitamin A and immune regulation: role of retinoic acid in gut-associated dendritic cell education, immune protection and tolerance. Mol Aspects Med. 2012;33:63–76.
6. Sun CM, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. J Exp Med. 2007;204:1775–85.
7. Coombes JL, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J Exp Med. 2007;204:1757–64.
8. Mucida D, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. Science. 2007;317:256–60.

9. Iwata M, et al. Retinoic acid imprints gut-homing specificity on T cells. Immunity. 2004;21:527–38.

10. Mora JR, et al. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. Science. 2006;314:1157–60.

11. Spencer SP, et al. Adaptation of innate lymphoid cells to a micronutrient deficiency promotes type 2 barrier immunity. Science. 2014;343:432–7.

12. Mielke LA, et al. Retinoic acid expression associates with enhanced IL-22 production by gammadelta T cells and innate lymphoid cells and attenuation of intestinal inflammation. J Exp Med. 2013;210:1117–24.

13. Bhattacharya N, et al. Normalizing microbiota-induced retinoic acid deficiency stimulates protective CD8(+) T cell-mediated immunity in colorectal cancer. Immunity. 2016;45:641–55.

14. Stockinger B, Di Meglio P, Gialitakis M, Duarte JH. The aryl hydrocarbon receptor: multitasking in the immune system. Annu Rev Immunol. 2014;32:403–32.

15. Li Y, et al. Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. Cell. 2011;147:629–40.

16. Kiss EA, et al. Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles. Science. 2011;334:1561–5.

17. Gandhi R, et al. Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell-like and Foxp3(+) regulatory T cells. Nat Immunol. 2010;11: 846–53.

18. Quintana FJ, et al. Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. Nature. 2008;453:65–71.

19. Veldhoen M, et al. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature. 2008;453:106–9.

20. Qiu J, et al. The aryl hydrocarbon receptor regulates gut immunity through modulation of innate lymphoid cells. Immunity. 2012;36:92–104.

21. Spann NJ, Glass CK. Sterols and oxysterols in immune cell function. Nat Immunol. 2013;14:893–900.

22. Parigi SM, Eldh M, Larssen P, Gabrielsson S, Villablanca EJ. Breast Milk and Solid Food Shaping Intestinal Immunity. Front Immunol. 2015;6:415.

23. Vavassori P, Mencarelli A, Renga B, Distrutti E, Fiorucci S. The bile acid receptor FXR is a modulator of intestinal innate immunity. J Immunol. 183:6251–61.

24. Cyster JG, Dang EV, Reboldi A, Yi T. 25-Hydroxycholesterols in innate and adaptive immunity. Nat Rev Immunol. 2014;14:731–43.

25. Hong C, Tontonoz P. Liver X receptors in lipid metabolism: opportunities for drug discovery. Nat Rev Drug Discov. 2014;13:433–44.

State-of-the-Art Lecture

IL-10-producing Tr1 regulatory cells: From the bench to the bedside

Maria Grazia Roncarolo[1], Rosa Bacchetta[1] and Silvia Gregori[2]
[1]Division of Stem Cell Transplantation and Regenerative Medicine, Department of Pediatrics, and ISCBRM, Stanford School of Medicine, Stanford, California, USA
[2]San Raffaele Telethon Institute for Gene Therapy (SR-TIGET), IRCCS San Raffaele Scientific Institute, Milan, Italy

Human $CD3^+CD4^+$ $FOXP3^+$ T regulatory (Treg), and T regulatory type 1 (Tr1) cells are essential for downregulation of immune and inflammatory responses and maintainance of immunological tolerance. Tr1 cells have an extrathymic origin and are not FOXP3 dependent for their immune regulatory function. Tr1 cells produce high levels of IL-10, TGF-β, low levels of IL-2 and variable amounts of IFN-γ, in the absence of IL-4 and IL-17 and express the co-cell surface receptors CD49b and LAG-3. IL-10 and TGF-β are indispensable for Tr1-mediated immunosuppression. Tr1 cells also kill myeloid cell lines via high granzyme B (GzB) expression induced by IL-10.
Due to their suppressive properties, T regulatory cells have been used as a cellular therapy for preventing excessive immune responses or abrogating autoimmune diseases. A number of clinical trials have been conducted with *in vitro* expanded Treg cells. We are currently testing human Tr1 cells in a clinical trial in patients receiving allogeneic hematopoietic stem cell transplantation (HSC). We have devised a method to produce allo Antigen-specific donor-derived Tr1 cells using $CD4^+$ T cells stimulated with host-derived tolerogenic DC (DC-10) as APC in the presence of IL-10 (T-allo10 cells). Their feasibility and safety is currently tested in an FDA approved Phase I clinical trial (IND# 17292) in patients receiving escalating doses of T-allo10 cells after mismatched related or unrelated unmanipulated HCT for hematologic malignancies.
A major question in this Treg cell therapy clinical approach are the stability of the Tr1 cells under inflammatory conditions and their *in vivo* homing capacity and survival. We investigated the use of lentiviral vector (LV) gene transfer to convert T effector (Teff) into Treg cells and to generate a stable and functional pool of Treg cells for clinical use. We showed that LV-mediated gene transfer of IL-10 in human $CD4^+$ T cells, converts human Teff cells into Tr1-like ($CD4^{IL\text{-}10}$) cells that express the Tr1 markers, suppress Teff cells and selectively kill myeloid cell lines and blasts in a HLA-class I- and Granzyme B-dependent manner *in vitro.* In a humanized model Tr1 cells protect from graft-versus-host disease while significantly delaying leukemia progression.

These findings provide a strong rationale for adoptive immunotherapy with Tr1 cells for the treatment of inflammatory and T-cell mediated disorders.

Session II

Microbiome

Diet and microbiota: Potential for therapeutic interventions

Stephan C. Bischoff
Institute of Clinical Nutrition, University of Hohenheim, Stuttgart, Germany

Diet is probably the most relevant regulator of the human intestinal microbiota. Besides other environmental factors, diet is a major contributor adding noise to quantitative measures of the microbiota in humans. Virtually all food components, in particular sugars and complex carbohydrates, shape the microbiota either in a beneficial or a harmful way. This shaping not only affects the composition of the microbiota but also the function. Energy harvest from food, generation of metabolites that might be toxic or warranted, and intestinal functions like motility and barrier integrity can be affected. Such mechanisms have implications for a number of diseases including GI diseases, liver diseases, obesity and obesity-associated metabolic diseases. Recent data revealed that diet-induced changes of microbiota diversity lasts for generations further emphasizing the potential of selected nutrients to shape the microbiota. Such data derived from mouse experiments motivate to consider nutrients that affect the microbiota in a preferable way for future therapeutic interventions. For example, diets rich in protein and fibers and containing low amounts of sugar have been proven beneficial for weight control, prevention of fatty liver disease, and prevention of colon carcinoma in individuals at risk. There is growing evidence that such effects are mediated, at least partially, the intestinal microbiota.

Neonatal imprinting of tolerogenic properties in gut-draining lymph nodes by microbiota

Jochen Huehn
Department Experimental Immunology, Helmholtz Centre for Infection Research, Braunschweig, Germany

The gastrointestinal tract constitutes the largest body surface and thus has developed various mechanisms to prevent pathogen entry and to efficiently eliminate invading pathogens. Simultaneously, the gastrointestinal system has to avoid unwanted immune responses against self and harmless non-self antigens, such as food- or microbiota-derived antigens. Accumulating evidence suggests that peripherally induced Foxp3$^+$ regulatory T cells (pTregs) play a key role in this process. We could recently demonstrate that gut-draining mesenteric lymph nodes (mLNs) show a higher *de novo* Treg-inducing capacity when compared to skin-draining peripheral LNs (pLNs). Interestingly, the high Treg-inducing capacity of mLNs was retained several weeks after their transplantation into the non-tolerogenic, skin-draining *popliteal fossa*, suggesting not only a so far largely unconsidered contribution of LN stromal cells in this process, but also demonstrating that the high Treg-inducing capacity of mLN stromal cells is stably imprinted and cannot be reverted by the skin microenvironment. Furthermore, transplantation of mLN from germfree and specific-pathogen free (SPF) mice revealed that microbiota are essentially required for this imprinting of the tolerogenic properties in mLN stromal cells. Data on the time point during ontogeny at which the imprinting process is taking place as well as on the microbial factors that educate mLN stromal cells for their site-specific function will be presented. In addition, specific emphasis will be laid on the modulation of functional properties of dendritic cells by mLN stromal cells and how this cross-talk is fostering the *de novo* generation of Tregs within the gastrointestinal immune system.

Regulation of mucosal immunity and barrier function by the intestinal microbiota

Till Strowig
Mikrobielle Immunregulation, Helmholtz Centre for Infection Research, Braunschweig, Germany

In healthy individuals, the microbiota and host maintain a symbiotic relationship, in which the microbiota provides benefits to the host by contributing to resistance against pathogens, to metabolic processes and to the proper development of the immune system. Notably, the composition of the microbiota can vary greatly among individuals being influenced by genetic predisposition and environmental factors, e.g. diet and use of antibiotics and other drugs. Numerous human disease conditions have been associated with imbalances in the composition of the gut microbiota, so-called dysbiosis, however, whether these changes contribute directly to the development of the disease or reflect an altered physiology of the host remains debated in many instances.
Inflammatory bowel disease is a group of heterogeneous diseases characterized by chronic and relapsing mucosal inflammation. Alterations in microbiota composition have been proposed to contribute to disease development, but no uniform signatures have yet been identified. In the last years we have compared the ability of a diverse set of microbial communities to exacerbate intestinal inflammation after chemical damage to the intestinal barrier. Strikingly, genetically identical wild type mice differing only in their microbiota composition varied strongly in their colitis susceptibility. Transfer of distinct colitogenic communities in gene-deficient mice revealed that they triggered disease via opposing pathways either independent or dependent on adaptive immunity, specifically requiring antigen-specific $CD4^+$ T cells. Our data provide evidence for the concept that microbial communities may alter disease susceptibility via different immune pathways despite eventually resulting in similar host pathology. This suggests a potential benefit for personalizing IBD therapies according to patient-specific microbiota signatures.

Lipid metabolism, microbiota and inflammation

Joerg Heeren
University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Adaptive thermogenesis is an energy-demanding process mediated by cold-activated beige and brown adipocytes, which requires increased uptake of dietary carbohydrates and lipids for maintaining caloric balance. The canonical lipid uptake pathway involves the hydrolysis of triglyceride-rich lipoproteins (TRL) by active lipoprotein lipase and the subsequent fatty acid uptake by active adipocytes. In addition to fatty acid uptake, we have shown whole TRL particle internalization into active brown adipose tissue (BAT).

Recently, we investigated in more detail the regulation as well as the molecular processes of lipid disposal into activated BAT, using pharmacological and genetic interventions in mice. We found that short-term BAT activation by cold exposure or beta-3-adrenergic receptor agonism triggers insulin secretion, a process depending on fatty acid release by white adipose tissue. Furthermore, we showed that both insulin release and brown adipocytes insulin sensitivity is essential for the replenishment of endogenous energy stores and efficient adaptive thermogenesis. Our data demonstrate that both catabolic and anabolic processes are important for energy balance and function of BAT.

In addition to increased fatty acid disposal, we found enhanced uptake of dietary cholesterol into activated BAT as consequence of lipoprotein internalization. Following the fate of cholesterol, we observed the induction of hepatic bile acid synthesis, interestingly via the alternative but not the classical pathway. This process, depending on hepatic CYP7B1 induction, results in elevated plasma levels and pronounced fecal excretion of conjugated bile acids, accompanied by distinct changes in gut microbiota. Pharmacological intervention using ezetimibe, a drug blocking dietary cholesterol uptake, prevented both the rise in bile acid excretion and compositional changes in gut bacteria in response to cold. These results identify bile acids generated in the liver as the determinant of cold-induced gut microbiota, highlighting the relevance of cholesterol metabolism by the host for diet-induced changes on gut microbiota.

Oral poster presentation

A novel mouse model to study IL-6/gp130 signaling and acute-phase proteins in the gut-liver axis

Neele Schumacher[1*], Miryam Müller[1], Karsten Lücke[2], Thomas Wunderlich[3], Hanna Lotter[4], Nicola Gagliani[5], Hans-Willi Mittrücker[2], Ateequr Rehman[6], Stefan Rose-John[1] and Dirk Schmidt-Arras[1]
[1]Institute of Biochemistry, Christian-Albrechts-University Kiel, Germany
[2]Institute of Immunology, University Medical Center Hamburg-Eppendorf, Germany
[3]Max-Planck Institute for Metabolism Research Cologne, Germany
[4]Department of Molecular Parasitology, Bernhard-Nocht Institute Hamburg, Germany
[5]Center for Internal Medicine, University Medical Center Hamburg-Eppendorf, Germany
[6]Institute of Clinical Molecular Biology, Christian-Albrechts-University Kiel, Germany
[*]E-Mail: nschumacher@biochem.uni-kiel.de

Introduction: Interleukin 6 (IL-6) is a pleiotropic cytokine involved in inflammation and tissue regeneration. In complex with its cognate IL-6 receptor it binds to the signal-transducing subunit gp130. In the liver and the gut, IL-6 has been shown to be crucial for the induction of acute-phase proteins (APP), liver regeneration, but also tumour formation. Recently, IL-6 was linked to microbial dysbiosis in a DSS colitis model.

Methods: We recently generated ROSA26-transgenic mice allowing for the Cre-inducible expression of a constitutive active gp130 variant (Lgp130). These mice were bred to Alb-CreERT2 or Vil-CreERT2 mice to allow tamoxifen-inducible expression of Lgp130 in hepatocytes or intestinal epithelial cells. Induction of APPs was analysed by qPCR and ELISA. Consequences of elevated APPs on inflammatory cells was assessed by flow cytometry and immunohistochemistry, whereas alterations in the gut microbiome were analysed by microbiome sequencing.

Results: We demonstrate that Lgp130 expression markedly induces expression of APPs either systemically (Alb-CreERT2) or locally (Vil-CreERT2). Systemic elevation of APPs by hepatocytic gp130 signals protects from *Listeria monocytogenes* infection, while intestinal gp130 signals altered the gut microbiome.

Discussion/Conclusion: Taken together, our model allows for the first time the analysis of gp130 signaling exclusively in hepatocytes or intestinal epithelial cells and its consequences on intestinal inflammation and the gut microbiome.

Oral poster presentation

Inhibition of gut microbiota can be a therapeutic target to reduce liver fibrosis

Fatma El Zahraa Ammar Mohamed[1,2#], Steven Dooley[1#], Nathan A. Davies[4], Seddik Hammad[1,3], Abeba Habtesion[4], Rajiv Jalan[4]
[1]Molecular Hepatology Section, Department of Medicine II, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany
[2]Department of Pathology, Faculty of Medicine, Minia University, Minia, Egypt
[3]Department of Forensic Medicine and Veterinary Toxicology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt
[4]UCL Institute for Liver and Digestive Health, Royal Free Hospital, London, UK
[#]Equal contribution
E-Mail: fatma.mohamed@medma.uni-heidelberg.de

Introduction: Despite of their beneficial effects, alterations of gut microbiota in liver diseases enhances endotoxins production. In addition, increased gut permeability in this setting maintains continuous liver exposure to gut-produced endotoxins, which in turn leads to persistent liver inflammation and fibrosis.

Aim of the work: Study the effect of reducing the endotoxin level – using the well-known anti-bacterial agent norfloxacin – on liver disease in a rat model of liver cirrhosis.

Methods: Eighteen Fisher rats divided into 3 groups; 1st treated with diethyl-nitrosamine (DEN) and oral N-nitrosomorpholine (NMOR). 2nd was treated with the same treatment + norfloxacin (20 mg/kg/day) from the beginning of the experiment to 14 weeks (end of experiment). 3rd group was served as a control. Endotoxin assay and liver functions were performed on animals' blood. H&E and reticulin were performed on liver tissue and TNF-α were measured.

Results: There was a significant reduction of endotoxin level ($p < 0.02$) and liver transaminases, namely ALT ($p < 0.05$) and AST ($p < 0.01$) in the norfloxacin treated group comparing to the DEN and NMOR group alone. With reticulin staining there was significantly reduction in liver fibrosis in the group treated with norfloxacin (score: 1 [0–2]) compared with the DEN and NMOR group (score: 4 [3–5]) ($p < 0.03$). There was also reduction of TNF-α level with norfloxacin treatment.

Conclusion: The results of this study suggest that selective decontamination of the gut by using norfloxacin leads to decrease circulating endotoxin may be a novel therapeutic strategy to treat fibrosis and enhance liver function.

Oral poster presentation

IgA antibodies against filamentous-actin are frequently detected in patients with cirrhosis and indicate a progressive disease course

Tamas Tornai[1], Boglarka Balogh[1], Nora Sipeki[1], Zsuzsanna Vitalis[1], Istvan Tornai[1], Peter Antal-Szalmas[2], Tamas Dinya[3], Gary L. Norman[4], Tony Bruns[5,6], Maria Papp[1*]
[1]Division of Gastroenterology, Department of Internal Medicine, [2]Department of Laboratory Medicine, [3]Institute of Surgery, Faculty of Medicine, University of Debrecen, Hungary; [4]Inova Diagnostics, Inc., San Diego, California, USA; [5]Department of Internal Medicine IV, University Hospital Jena, Germany; [6]Center for Sepsis Control and Care, University Hospital Jena, Germany
[*]E-Mail: drpappm@yahoo.com

Introduction: Gut barrier failure and the consequential pathological bacterial translocation (BT) are characteristic features of cirrhosis and play an important role in the progression of liver disease. We hypothesized that serological hallmarks of gut barrier dysfunction are associated with accelerated progression of liver disease in cirrhosis and the development of specific complications and liver-related death.

Methods: Sera from 260 stable outpatients with cirrhosis (male: 129, age: 56 ± 11 years, alcohol: 167 [64.2%]) and from 155 healthy subjects were assayed for the presence of antibodies against filamentous-actin (AAA IgA and IgG) and gliadin (AGA IgA and IgG) and for intestinal fatty acid-binding protein (I-FABP) by ELISA. Association of gut failure markers with disease specific characteristics was assessed at baseline and the course of liver disease was evaluated in a 5-year follow-up observational study for decompensating events (ascites, variceal bleeding, hepatic encephalopathy and/or bacterial infection) and liver-related death. BT was assessed based on the presence of anti-microbial antibodies (anti-OMP plus IgA and/or endotoxin core IgA antibody [EndoCab]).

Results: Elevated concentrations of the gut failure markers IgA-AAA (62.7 vs. 4.4%) and IgA-AGA (27.7 vs. 2.6%) were more often observed in cirrhosis as compared to healthy controls ($p < 0.001$ for both). In addition, serum I-FABP was increased in cirrhosis as compared to controls (741 vs. 244 pg/ml, $p < 0.001$) and correlated with serum levels of IgA-AAA and IgA-AGA. IgA-AAA positivity was associated with alcoholic liver disease, liver disease scores and decompensated clinical stage (all $p < 0.001$). Serological markers of BT were more often found in patients with elevated IgA-AAA compared to those without (72.3 vs. 13.5 % for IgA-EndoCab and 85.2 vs. 20.5% for IgA-anti-OMP, $p < 0.001$ for both). In patients with compensated disease stage ($n = 131$) the risk of decompensation was higher in patients with elevated IgA-AAA (HR = 1.85; 95% CI: 1.06–3.24), as was the risk of liver-related mortality (HR = 2.66; 95% CI: 1.27–5.56). Such associations were not observed for IgG-AAA and IgA/IgG-AGAs. In the overall cohort, IgA-AAA remained an independent predictor

of liver-related death (HR_{adj} = 1.96; 95% CI: 1.08–3.55]) when adjusting for important clinical variables (MELD score, etiology, clinical stage, see **Table 1**).

Table 1. Summary of Cox proportional hazards analysis in the prediction of liver-related mortality.

n = 260	Wald	Hazard ratio	95% confidence interval	p-value
AAA IgA positivity (≥ 35 U)	4.83	1.96	(1.08–3.58)	0.028
MELD score (per 1 point increase)	11.71	1.10	(1.04–1.17)	0.001
Etiology (alcohol)	1.63	0.70	(0.40–1.21)	0.202
Clinical stage (decompensated)	1.73	1.38	(0.86–2.22)	0.188

Discussion/Conclusion: Elevated serum concentrations of IgA antibodies against filamentous-actin indicate patients with an unfavourable outcome in cirrhosis, which may be related to intestinal damage beyond being related to bacterial translocation. IgA-AAA might be consider as a novel serologic marker of the disease progression.

Oral poster presentation

L-selectin (CD62L) is increased in patients with ulcerative colitis and drives the progression of non-alcoholic steatohepatitis (NASH) in mouse and men

H.K. Drescher[1*], A. Schippers[2], H. Sahin[1], C. Trautwein[1], D.C. Kroy[1]
[1]Department of Internal Medicine III, University Hospital RWTH Aachen
[2]Department of Paediatrics, University Hospital RWTH Aachen, Germany
[*]E-Mail: hdrescher@ukaachen.de

Introduction: NASH is one of the fastest growing medical problems. The significance of the CD62L dependent lymphocyte infiltration is known in inflammatory bowel disease. Studies show that CD62L/MAdCAM-1-dependent cell-infiltration induces chronic colitis. In contrast, the role of CD62L in NASH remains unclear. Our current study investigates CD62L in patients with steatosis and in two mouse-steatohepatitis models.

Methods: Expression of soluble L-selectin (sL-selectin) was analysed in serum of patients with acute and chronic liver diseases. Hepatic expression of L-selectin was measured in patients with steatosis and NASH. Furthermore, constitutive L-selectin$^{-/-}$ mice were fed MCD-diet (methionine and choline deficient) for 4 weeks or HF-diet (high fat) for 24 weeks.

Results: Patients with NASH and acute liver injury display increased serum levels of sL-selectin. Interestingly serum levels also increase in patients after treating ulcerative colitis (UC) with vedolizumab but not in UC patients per se. Hepatic expression of CD62L is higher in patients with steatosis and increases dramatically in NASH patients. Coherent with the human data, MCD and HFD treated CD62L$^{-/-}$ mice showed a less invasive phenotype. This attenuated disease pathogenesis was reflected less fatty liver degeneration. Furthermore, CD62L$^{-/-}$ animals displayed a dampened manifestation of the metabolic syndrome with an improved insulin resistance and decreased levels of cholesterol and triglycerides in both steatohepatitis models. The amelioration of steatohepatitis was further reflected by lower transaminases. Consistent with the less invasive phenotype, CD62L$^{-/-}$ animals showed an enhanced hepatic infiltration of T_{Reg} cells. Those changes finally resulted in a protection of CD62L$^{-/-}$ mice from progression to liver fibrosis.

Discussion/Conclusion: CD62L is increased in patients with fatty liver disease. To analyse the underlying mechanisms, we could show that CD62L deficiency in mice leads to a protection against diet induced steatohepatitis. Therefore, the blockade of CD62L provides a novel target for therapeutic interventions during NASH.

Session III

Metabolism and inflammation

Fatty liver disease: Role model of immunometabolic diseases?

Frank Tacke
Department of Medicine III, University Hospital Aachen, Aachen, Germany; E-Mail: ftacke@ukaachen.de

Non-alcoholic fatty liver disease (NAFLD) and its progressive inflammatory form, non-alcoholic steatohepatitis (NASH), are leading causes of liver cirrhosis and hepatocellular carcinoma. Metabolism and inflammation are intimately interrelated in NAFLD/NASH, as expressed by the term immunometabolism. Macrophages represent a key cellular immunological component of the liver that ensures homeostasis and rapid responses to hepatic injury. Thereby, hepatic macrophages mediate inflammatory responses during metabolic disorders and can stimulate or dismantle liver fibrosis. Their functional diversity is partly explained by heterogeneous macrophage subsets, i. e. tissue-resident Kupffer cells and monocyte-derived macrophages. However, macrophages themselves are altered in their functional polarization by dietary composition, metabolic or inflammatory stimuli in NAFLD. The inflammatory polarization of macrophages correlates with changes in core metabolism pathways like oxidative phosphorylation and glycolysis. The availability of nutrients, such as glucose or fatty acids, or oxygen also influences macrophage polarization upon danger signal or cytokine reception. Understanding the interplay of metabolism and macrophage function in NASH may open new approaches to therapeutic targeting of these essential modifiers in metabolic liver diseases. Studies are ongoing to pharmacologically inhibit inflammatory monocyte influx into the liver or to augment the differentiation of restorative macrophages. The oral dual chemokine receptor CCR2/CCR5 antagonist cenicriviroc significantly inhibits the accumulation of monocyte-derived inflammatory macrophages in animal models of liver injury, thereby ameliorating experimental steatohepatitis and hepatic fibrosis. This concept is currently being evaluated in clinical trials in patients with NASH and fibrosis. The emerging basic experimental, translational and early clinical data support the concept of fatty liver disease as a role model of an immunometabolic disease, which forms the basis for novel therapeutic interventions.

State-of-the-Art Lecture

How to maintain liver immune tolerance

Percy A. Knolle, MD
Molekulare Immunologie, Klinikum rechts der Isar der Technischen Universität, Munich, Germany

The liver is known for its ability to induce immune tolerance, for which the unique hepatic microenvironment and its organ-resident antigen-presenting cell populations are considered to be important. The identification of the molecular mechanisms governing hepatic immune tolerance is highly relevant for the understanding and treatment of liver autoimmunity, persistent viral infections of the liver and liver transplant rejection. Beyond the identification of cells and molecular mechanisms involved in immune tolerance in the liver, knowledge on the relative strengths of these tolerogenic mechanisms in the context of local and systemic immune responses is required to predict the outcome of an immune response. Liver immune tolerance therefore rather appears to be defined as operational tolerance than resulting from absolute deterministic tolerogenic pathways.

Session IV

Liver inflammation

Type I IFN in liver fibrosis; an indisputable harassing factor

Z. Abdullah
Institut für Experimentelle Immunologie, Universitätsklinikum Bonn, Germany

A common clinical complication in patients suffering from chronic liver inflammation and fibrosis is enhanced susceptibility and exacerbations to viral infections as well as weak responses to vaccination. To unravel the cellular and molecular underpinnings of the impaired cell mediated immunity, we investigated the immune responses to LCMV as well as *Listeria* infections in the context of liver fibrosis. Using preclinical models of liver fibrosis, i.e. bile duct ligation and carbon tetrachloride application we found that chronic liver damage is associated with loss of T cell immunity and enhanced susceptibility to LCMV and *Listeria* infections leading to persistence infection, recapitulating the clinical situation in humans. Hallmarks of this failure to mount protective immunity are reduced numbers and malfunction of antigen-specific CD4 and CD8 T cells that express high levels of inhibitory receptors. Mechanistically, we identified continuous IFN receptor (IFNAR) signaling in myeloid cells triggered by gut microbiota as the basis for induction of T cell exhaustion after viral infection through IL-10 production by myeloid cells. Strikingly, we could reconstitute protective immunity and promote infection clearance by either blocking IFNAR signaling in myeloid cells, abrogate the IFNAR-induced IL-10 production or by systemic blockade of IL-10 receptor signaling. We have validated these findings using *in vitro* stimulation assay of T cells from alcoholic- liver cirrhosis patients vaccinated against HBV and HAV. Thus, our results reveal the key role of type I interferon in myeloid cells as molecular trigger and IL-10 as execution mechanism responsible for restraint T cell immunity. Our results identify an exciting new therapeutic avenue for treatment of infections and to enhance vaccination efficacy in patients with chronic liver damage and cirrhosis.

Adaptive immune cells and liver inflammation

M. Iannacone
Division of Immunology, San Raffaele Scientific Institute, Milan, Italy

$CD8^+$ T cells have a key role in eliminating intracellular pathogens and tumors that affect the liver. The protective capacity of these cells relies on their ability to migrate to and traffic within the liver, recognize pathogen- or tumor-derived antigens, get activated and deploy effector functions. While some of the rules that characterize $CD8^+$ T cell behavior in the infected and cancerous liver have been characterized at the population level, we have only limited knowledge of the precise dynamics of intrahepatic $CD8^+$ T cell conduct at the single-cell level. In the last few years, we have developed several advanced imaging techniques that allow us to dissect the interactive behavior of $CD8^+$ T cells within the mouse liver at an unprecedented level of spatial and temporal resolution. This approach, combined with unique models of hepatitis B virus pathogenesis and a new model of hepatocellular carcinoma created *ad hoc*, has generated novel mechanistic insights into the spatiotemporal determinants that govern the capacity of $CD8^+$ T cells to home and function in the virus- or tumor-bearing liver. We will discuss here our recent results on how the anatomical, hemodynamic and environmental cues that characterize hepatocellular carcinomas shape $CD8^+$ T cell behavior and function; we will also provide a detailed characterization of intrahepatic T cell priming events that induce functionally defective T cell responses. This new knowledge may lead to improved vaccination and treatment strategies for immuno-therapy of infectious diseases and cancer.

List of Chairpersons, Speakers and Scientific Organizers

Dr. Zeinab Abdullah
Institut für Experimentelle Immunologie
Universitätsklinikum Bonn
Sigmund-Freud-Str. 25
53127 Bonn
Germany

Prof. Dr. David H. Adams
Liver Research Laboratories
Institute of Clinical Sciences
Queen Elizabeth Hospital
Birmingham B15 2TH
Great Britain

Prof. Dr. Stephan C. Bischoff
Institut für Ernährungsmedizin
Universität Hohenheim
Fruwirthstr. 12
70599 Stuttgart
Germany

Prof. Dr. Johannes G. Bode
Klinik für Gastroenterologie,
Hepatologie und Infektiologie
Universitätsklinikum Düsseldorf
Moorenstr. 5
40225 Düsseldorf
Germany

Prof. Dr. Eran Elinav
Department of Immunology
Weizmann Institute of Science
Wolfson Bldg., Room 211
Rehovot 7610001
Israel

Prof. Dr. Andre Franke
Klinische Molekularbiologie
Christian-Albrechts-Universität
Rosalind-Franklin-Str. 12
24105 Kiel
Germany

Prof. Dr. Nicola Gagliani
I. Medizinische Klinik und Poliklinik
(Gastroenterologie mit Sektionen
Infektiologie und Tropenmedizin)
Zentrum für Innere Medizin
Universitätsklinikum Hamburg-Eppendorf
Martinistr. 52
20246 Hamburg
Germany

Prof. Dr. Jörg Heeren
Institut für Biochemie und
Molekulare Zellbiologie
Zentrum für Experimentelle Medizin
Universitätsklinikum Hamburg-Eppendorf
Martinistr. 52
20246 Hamburg
Germany

Prof. Dr. Samuel Huber
I. Medizinische Klinik
Universitätsklinikum Hamburg-Eppendorf
Martinistr. 52
20246 Hamburg
Germany

Prof. Dr. Jochen Huehn
Experimentelle Immunologie
Helmholtz-Zentrum für
Infektionsforschung
Inhoffenstr. 7
38124 Braunschweig
Germany

Dr. Dr. Matteo Iannacone
Division of Immunology
San Raffaele Scientific Institute
Via Olgettina 58
20132 Milan
Italy

Prof. Dr. Percy A. Knolle
Molekulare Immunologie
Klinikum rechts der Isar
der Technischen Universität
Ismaninger Str. 22
81675 München
Germany

Prof. Dr. Ansgar W. Lohse
I. Medizinische Klinik
Universitätsklinikum Hamburg-Eppendorf
Martinistr. 52
20246 Hamburg
Germany

Prof. Dr. Markus F. Neurath
Medizinische Klinik I
Universitätsklinikum Erlangen
Ulmenweg 18
91054 Erlangen
Germany

Prof. Dr. Maria G. Roncarolo
Division of Stem Cell Transplantation
and Regenerative Medicine
Department of Pediatrics
Stanford University
Lorry I. Lokey Stem Cell
Research Building
265 Campus Drive
Stanford, CA 94304-5454
USA

Prof. Dr. Christoph Schramm
I. Medizinische Klinik
Universitätsklinikum Hamburg-Eppendorf
Martinistr. 52
20246 Hamburg
Germany

Prof. Dr. Andreas Stallmach
Klinik für Innere Medizin IV
(Gastroenterologie, Hepatologie,
Infektiologie, Interdisziplinäre
Endoskopie)
Universitätsklinikum Jena
Am Klinikum 1
07747 Jena
Germany

Dr. Till Strowig
Mikrobielle Immunregulation
Helmholtz-Zentrum für
Infektionsforschung
Inhoffenstr. 7
38124 Braunschweig
Germany

Prof. Dr. Frank Tacke
Medizinische Klinik III
Universitätsklinikum Aachen
Pauwelsstr. 30
52074 Aachen
Germany

Prof. Dr. Robert Thimme
Klinik für Innere Medizin II
Universitätsklinikum Freiburg
Hugstetter Str. 55
79106 Freiburg
Germany

Prof. Dr. Herbert Tilg
Universitätsklinik für Innere Medizin I
Medizinische Universität Innsbruck
Anichstr. 35
6020 Innsbruck
Austria

Prof. Dr. Christian Trautwein
Medizinische Klinik III
Universitätsklinikum Aachen
Pauwelsstr. 30
52074 Aachen
Germany

Prof. Dr. Eduardo J. Villablanca
Department of Medicine (Solna)
Immunology and Allergy Unit
Karolinska Institute, L2:04
171 76 Stockholm
Sweden

Prof. Dr. Jan Wehkamp
Innere Medizin I
Universitätsklinikum Tübingen
Otfried-Müller-Str. 10
72076 Tübingen
Germany

POSTER ABSTRACTS

Poster Numbers 1 – 48

Author Index to Poster Abstracts

Morpho-functional changes of lymphoid thymus cells on the background of prolonged use of proton-pump inhibitors

A.V. Antonenko[1], S.V. Pylypenko[2], O.H. Korotkyi[3], T.V. Beregova[3], L.I. Ostapchenko[3]
[1]Bogomolets National Medical University, Department of Internal Medicine 3
[2]Poltava National V.G. Korolenko Pedagogical University, Department of Biology and Basis of Human Health, Poltava, Ukraine
[3]Taras Shevchenko National University of Kiev, Ukraine

Introduction: Under the influence of physical and chemical factors, especially prolonged intake of proton-pump inhibitors, morpho-functional changes of thymus can be noted, which lead to the transformation of chronic inflammatory process in the stomach and colon into the tumour. The aim of our work was to investigate the morpho-functional status of lymphoid thymus cells in rats under prolonged inhibition of gastric secretion by omeprazole (OM) and in the case of long-term co-administration of OM and multiprobiotics as a way of preventing inflammation in the digestive tract, and also to determine the production of interferon (IFN) by lymphoid cells of thymus, which has a leading role in the body's immune response.

Methods: The study was done on 30 white rats which were divided into 3 groups. Animals of the 1st group (control) were intraperitoneally (i.p.) injected with 0.2 ml of H_2O. Animals of the 2nd group were injected with omeprazole OM (14 mg/kg, diluted in 0.2 ml of H_2O, i.p.). Animals of the 3rd group were simultaneous injected with OM and multiprobiotic “Symbiter® acidophilic” concentrated. All drugs were injected during 28 days. The reaction of the immune system's response was evaluated with the study of the weight and cellularity of the lymphoid organs.

Results: In rats in the control group the weight and cellularity of the thymus were $25 \pm 2.2 \times 10^{-4}$ and $60 \pm 5.5 \times 10^{7}$ CU, respectively. After 28 days of OM administration the relative weight of the thymus decreased by 40% ($p < 0.05$). The reduction of the relative weight of thymus occurred simultaneously with increasing of the relative content of lymphoid cells in it by 53.3% ($p < 0.05$) compared to control. The 28-day co-administration of OM and multiprobiotic resulted in an increase of the relative weight of thymus by 20% ($p < 0.05$) compared with the group of rats that received only OM. Relative cellularity of thymus over 28 days in the 2nd group, was on 30.4% ($p < 0.05$) higher than in the 3rd group and 100% ($p < 0.05$) higher than in the control group. Introduction during 28 days of OM led to an increase in spontaneous IFN production of thymocytes by 156.4% ($p < 0.05$) compared to control.

Discussion/Conclusion: An increase of the relative content of lymphoid cells in the thymus after prolonged inhibition of the secretion of HCl in the stomach of rats is a reflection of the activation of proliferative processes in the thymus. This suggests that such thymus response is the result of activating cellular immunity on the background of dysbiosis, requiring the additional number of T-lymphocytes. Reducing the relative weight of the thymus indicates its degradation.

2

NAFLD and intestinal microbiota: Clinical parallels

I. Bakulin, M.P. Abatsieva, L.N. Belousova
Propaedeutic of Internal Diseases Gastroenterology, North-Western State Medical University n.a. I.I. Mechnikov, Saint Petersburg, Russia

Assumptions about the relationship between fatty hepatosis and intestinal dysbiosis have been expressed for a long time, but the study of the relationship between the microbiota and the severity of NAFLD is still of clinical interest. Under conditions when numerous mechanisms supporting the endogenous microflora of the intestine turn out to be untenable, conditions are created for the translocation of intestinal bacteria. Endotoxins of microorganisms damage the cell membranes, disrupt the processes of ion transport, induce the formation of free radicals. The latter play a key role in the formation of steatohepatitis.

Introduction: Analysis of current data on the effect of intestinal microbiota on the development and progression of NAFLD.

Methods: A review of PubMed data was carried out using the following key words: "NAFLD", "NASH", "steatosis", "intestinal microbiota" and "microbiomas".

Results: In a randomized clinical trial of patients with histologically confirmed NAFLD (Wong VW, et al. 2015), the intrahepatic triglyceride content was studied by proton magnetic resonance spectrometry against a background of a six-month administration of a multi-stencil probiotics complex containing lactobacilli and bifidobacteria, received a probiotic complex, the level of triglycerides in the liver decreased by 30% ($p = 0.034$) compared with patients in the control group ($p = 0.55$), and also in the study group A more significant decrease in AST activity was observed. Boursier J, et al. (2016) by the method of sequencing ribosomal RNA genes 16S studied the taxonomic composition of the intestinal microbiota in patients with NAFLD, depending on the stage of fibrosis (according to METAVIR). Multivariate analysis showed that excess bacterial growth with a decrease in the level of Prevotella is independently associated with the presence of NASH, an increase in the amount of Ruminococcus is associated with $F \geq 2$.

Discussion/Conclusion: The severity of NAFLD is associated with a change in the metabolic function of the intestinal microbiota and the severity of dysbiosis. The analysis of the intestinal microbiota adds information to the classical predictors of severity of NAFLD and offers new metabolic targets for treatment.

The influence of an altered microbiota and PSC on colitis severity

Simon Bohmann, Tanja Bedke, Cathleen Haueis, Nicola Gagliani, Samuel Huber
Universitätsklinikum Hamburg-Eppendorf, Germany

Introduction: Approximately 70% of primary sclerosing cholangitis (PSC) patients show a concomitant inflammatory bowel disease (IBD), whereas only 2–7% of IBD patients suffer from PSC. The reason for this association is currently unclear. One possibility is that PSC affects colitis development via alterations of the microbial and bile acid composition that in turn exacerbates liver pathology. In accordance, it has been shown in germ-free *Mdr2*$^{-/-}$ mice, a mouse model of experimental PSC, that the intestinal microbiota is necessary to control liver pathology in these mice.

Objectives: In this project, we aimed to investigate the influence of PSC on spontaneous or induced colitis development in the presence of a dysbiotic intestinal microbiota in *Mdr2*$^{-/-}$ mice.

Methods: For this purpose, we transferred dysbiotic and control faeces into *Mdr2*$^{-/-}$ mice as well as wild-type littermate controls and assessed colitis severity before and after induction of DSS colitis.

Results: Five weeks after transplantation, dysbiotic microbiota was detectable in the stool of *Mdr2*$^{-/-}$ mice and wild-type littermates as indicated by the presence of *prevotella* mRNA. In accordance, these mice developed a mild spontaneous colitis whereas no signs of colitis development were detectable in mice that had received symbiotic control faeces. As expected, wild-type mice that had received dysbiotic faeces developed more severe DSS colitis than wild-type mice that had received control faeces. Surprisingly, *Mdr2*$^{-/-}$ mice with dysbiotic microbiota were protected from DSS-colitis development compared to wild-type littermates.

Conclusion: The intestinal microbiota plays a crucial role for the susceptibility of colitis in mice. Thus, transfer of a dysbiotic microbiota promoted mild spontaneous colitis in *Mdr2*$^{-/-}$ mice and wild-type littermates. More importantly, our data indicate that PSC might protect from the development of severe DSS-colitis in mice with a dysbiotic microbiota. These data suggest that the intestinal microbiota might play a role in counter-regulating exacerbation of colitis in PSC patients. However, future studies are necessary to understand the underlying mechanisms.

4

Mechanisms how gut-derived bacterial toxin (LPS) affects liver cells

Haristi Gaitantzi, Chen Cai, Matthias Ebert, Katja Breitkopf-Heinlein
II. Medical Clinic, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

Introduction: Lipopolysaccharide (LPS) is a bacterial toxin in the cell membrane of gram-negative bacteria. Dysregulations of the bacterial gut flora and/or damage to the gut mucosa lead to a extravasation of LPS to the blood stream resulting in translocation to the liver. The LPS receptor TLR4 is expressed on several different liver cell types including the liver macrophages (Kupffer cells; KC). While responses of these cells have been studied quite intensively already, direct effects of LPS on other liver cell types like liver sinusoidal endothelial cells (LSEC) or hepatic stellate cells (HSC) were much less addressed up to now and were therefore the focus of the present investigation.

Methods: Primary isolated liver cell types or cell lines from mice or human were used and expression levels of TLR4 and other immune-response related genes or growth factors were investigated by real time PCR.

Results: When comparing TLR-4 expression in primary hepatocytes, HSC, LSEC or KC we found highest expression in KC, as expected. LSEC however also expressed substantial amounts of this receptor whereas TLR4 expression in HSC was low and not detectable in hepatocytes. Although treating HSC with LPS did not lead to enhanced activation towards a myofibroblastic phenotype it strongly down-regulated expression of the pro-fibrogenic growth factor BMP-9. BMP-9 on the other hand induced expression of TLR4 in LSEC, implying that under fibrotic conditions (where BMP-9 secretion from HSC is enhanced) LSEC become more sensitive to LPS by upregulating TLR4.

Discussion/Conclusion: We conclude that there exists a fine-tuned regulatory cross-talk between HSC and LSEC that at least partially controls the hepatic responses to LPS.

L-selectin (CD62L) is increased in patients with ulcerative colitis and drives the progression of non-alcoholic steatohepatitis (NASH) in mouse and men

H.K. Drescher [1], A. Schippers[2], H. Sahin[1], C. Trautwein[1], D.C. Kroy[1]
[1]Department of Internal Medicine III, University Hospital RWTH Aachen
[2]Department of Paediatrics, University Hospital RWTH Aachen, Germany

Introduction: NASH is one of the fastest growing medical problems. The significance of the CD62L dependent lymphocyte infiltration is known in inflammatory bowel disease. Studies show that CD62L/MAdCAM-1-dependent cell-infiltration induces chronic colitis. In contrast, the role of CD62L in NASH remains unclear. Our current study investigates CD62L in patients with steatosis and in two mouse-steatohepatitis models.

Methods: Expression of soluble L-selectin (sL-selectin) was analysed in serum of patients with acute and chronic liver diseases. Hepatic expression of L-selectin was measured in patients with steatosis and NASH. Furthermore, constitutive L-selectin$^{-/-}$ mice were fed MCD-diet (methionine and choline deficient) for 4 weeks or HF-diet (high fat) for 24 weeks.

Results: Patients with NASH and acute liver injury display increased serum levels of sL-selectin. Interestingly serum levels also increase in patients after treating ulcerative colitis (UC) with vedolizumab but not in UC patients per se. Hepatic expression of CD62L is higher in patients with steatosis and increases dramatically in NASH patients. Coherent with the human data, MCD and HFD treated CD62L$^{-/-}$ mice showed a less invasive phenotype. This attenuated disease pathogenesis was reflected less fatty liver degeneration. Furthermore, CD62L$^{-/-}$ animals displayed a dampened manifestation of the metabolic syndrome with an improved insulin resistance and decreased levels of cholesterol and triglycerides in both steatohepatitis models. The amelioration of steatohepatitis was further reflected by lower transaminases. Consistent with the less invasive phenotype, CD62L$^{-/-}$ animals showed an enhanced hepatic infiltration of T_{Reg} cells. Those changes finally resulted in a protection of CD62L$^{-/-}$ mice from progression to liver fibrosis.

Discussion/Conclusion: CD62L is increased in patients with fatty liver disease. To analyse the underlying mechanisms, we could show that CD62L deficiency in mice leads to a protection against diet induced steatohepatitis. Therefore, the blockade of CD62L provides a novel target for therapeutic interventions during NASH.

6

Non-invasive diagnostic of liver fibrosis in patients with type 2 diabetes with complications in daily practice

Karel Dvorak, Yassin Barbora, Anna Hovorkova, Jindra Vejrychova[+]
Department of Gastroenterology and Hepatology
[+]Department of Diabetology
Krajská Nemocnice Liberec, Czech Republic

Introduction: The amount of liver fibrosis represents the most important prognostic factor in NAFLD. We aimed to assess the possibilities of non-invasive staging of liver fibrosis in difficult to examine patients.

Methods: 93 consecutive patients with type 2 diabetes (DM2) with late complications were examined (basic laboratory examination, anthropometric parameters, abdominal ultrasound with point-shear wave elastography). Non-invasive scores of liver fibrosis (APRI, FIB-4, BARD, NAFLD fibrosis score) were calculated.

Results: We examined 93 patients (mean age 68 ± 10.1 years, BMI 30.1 ± 4.58, waist circumference 110 ± 11.1 cm, none patient had normal waist circumference, all had metabolic syndrome). Steatosis was present in 65 (70%) patients, elastography measurement meeting quality criteria was possible in 56 (60%) patients, borderline in 17 (18.2%), not possible in 20 (21.5%) patients. 7 patients (9.6%) of the reliable/ borderline measurable group had values in a range of advanced fibrosis/cirrhosis (F3/4). The reliability of measurement was associated with lower BMI, waist circumference and thoracic wall thickness. In the group of patients which could not have been measured the non-invasive scores were of no use, because they brought controversial results.

Discussion/Conclusion: Type 2 diabetic patients with late complications represent a subgroup of patients with significant prevalence of advanced liver fibrosis/cirrhosis. In a substantial amount of these patients elastography measurement is unreliable and basic non-invasive scoring systems are of no help.

Analysis of murine and human histone deacetylase 7 expression in fibrosis and its subcellular localization during inflammation

Kim Freese, Judith Sommer, Claus Hellerbrand
Institute of Biochemistry, Friedrich-Alexander University of Erlangen-Nürnberg, Erlangen, Germany

Introduction: Recent evidence has highlighted a pathological imbalance in hepatic fibrosis between the acetylation and deacetylation of histone proteins regulated by histone deacetylases (HDACs). More recently, HDAC inhibition appeared as therapeutic strategy in hepatic fibrosis and hepatocellular cancer. Mostly unspecific HDAC inhibition (HDACi) has been applied and the knowledge about the expression and role of individual HDACs during the progression of chronic liver disease is very limited.
Previously we could show a novel link between CYLD and HDAC7 contributing to progression of liver disease in hepatocellular damage and liver fibrosis.
The aim of the study was to unveil the role of HDAC7 during liver disease and to evaluate firstly the expression level of HDAC7 in different liver fibrosis models as well as its subcellular localisation during inflammation.

Methods and results: HDAC7 mRNA expression from murine samples from high-fat diet fed mice didn't show changes at mRNA expression level in qPCR analysis. Mice fed a high-fat diet and additional fructose in drinking water, developed more prominent fibrosis and showed 5-fold increase in HDAC7 expression. Further, HDAC7 expression increased 3-fold compared to controls in liver tissue samples from mice fed a so called Paigen diet, consisting of high fat, high cholesterol and cholate, inducing mild fibrosis. In the liver samples of methionine- and choline-deficient diet, another well-established fibrosis model, we also found 3-fold increased HDAC7 levels.
After treatment of LX2, a hepatic stellate cell cell line, with TNF-alpha, a pro-inflammatory cytokine, HDAC7 unveiled changes in subcellular localization which was analyzed with immunofluorescence.

Discussion/Conclusion: During liver fibrosis HDAC7 expression levels appear to generally increase, whereas simple steatosis doesn't affect HDAC7 mRNA expression. To determine the cell type which is responsible for this increase we initially focused on HSC as they undergo activation during fibrosis and thereby could verify correlation between collagen and HDAC7 expression. Further, the subcellular localization of HDAC7 during inflammation could play an important role for downstream events of HDAC7. The reported changes in HDAC7 expression in healthy and pathologic state can contribute to the understanding of the role of HDAC7 in the pathology of hepatic fibrosis and the development and progression of hepatocellular carcinoma. Further making it a specific target for HDACi treatment in liver disease therapy.

8

A novel MAPK14-ATF2-microRNA axis contributes to hepatocellular carcinoma progression and sorafenib resistance

Valerie Fritz[1], Lara Malek[1], Claus Hellerbrand[1,2], Anja Katrin Bosserhoff[1,2], Peter Dietrich[1]
[1]Institute for Biochemistry, Emil-Fischer-Zentrum, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany
[2]Comprehensive Cancer Center (CCC) Erlangen-EMN, Erlangen, Germany

Introduction: Chronic liver damage/inflammation are hallmarks of hepatocellular carcinoma (HCC) development and progression. The multi-kinase inhibitor sorafenib is the only effective therapeutic option for advanced HCC. However, the precise role of the stress- and inflammation-induced *mitogen-activated kinase 14* (MAPK14) is unknown in HCC progression and therapy resistance. We aimed at exploring the expression, function, and regulation of MAPK14 in HCC progression and resistance to sorafenib.

Methods: Primary human hepatocytes (PHH) and human HCC cell lines (Hep3B, HepG2, PLC, Huh-7) as well as sorafenib-resistant Hep3B cells were analyzed. Patient samples derived from HCC and corresponding non-tumorous liver tissues were used for *in vivo* quantification of mRNA levels. MicroRNA mimics (miR-622) and si-RNA-pools targeting the MAPK14 and *activating transcription factor 2* (ATF2) mRNAs were used for functional analysis.

Results: MAPK14 was overexpressed in HCC cell lines and in HCC patient-derived tissue samples. MAPK14 expression was found to be regulated by the novel downregulated tumor suppressive miR-622. Interestingly, MAPK14 can lead to activation of ATF2, which was also identified as a target gene of miR-622 in HCC in this study. MAPK14 and ATF2 mRNA and protein levels were significantly downregulated after re-expression of miR-622. Moreover, luciferase reporter assays revealed that specific 3'UTR *MicroRNA Response Elements* in the MAPK14 and ATF2 mRNA transcripts bind miR-622. Both MAPK14 and ATF2 were upregulated in sorafenib-resistant cell lines as compared to non-resistant cells and synergistically induced drug resistance in these cells.

Discussion/Conclusion: We found a novel miR-622-MAPK14-ATF2 axis in HCC. Both MAPK14 and ATF2 are overexpressed in HCC and contribute to sorafenib-resistance. MiR-622 is a downregulated and tumor suppressive microRNA that was newly described to target both MAPK14 and ATF2 in HCC, revealing a novel gene crosstalk with potentially therapeutic effects.

Human 3-dimensional intestinal and liver organoids: In vitro model systems to study the gut-liver axis

Haristi Gaitantzi, Chen Cai, Niklas Rindtorff, Johannes Betge, Matthias Ebert, Katja Breitkopf-Heinlein
II. Medical Clinic, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany
Signaling and Functional Genomics/B110, German Cancer Research Center (DKFZ), Heidelberg, Germany

Introduction: There is by now a common agreement that 2-dimensional, monolayer cell cultures, even if they are composed of primary isolated cells, do not sufficiently reflect the in vivo situation. Therefore emerging effort is created to generate 3-dimensional tissue culture models, so called organoids. Accordingly, the goal of the present study is to generate gut organoids from human colon biopsies and liver organoids from quasi primary human liver cells and to use these models to study gut-liver interactions in vitro.

Methods: According to published protocols we isolated primary fibroblasts and crypts of the intestinal mucosa from fresh human biopsy tissue samples. Liver organoids were formed by spontaneous assembly of human hepatocytes, LSEC (liver sinusoidal endothelial cells) and HSC (hepatic stellate cells) seeded in a physiological ratio of 70%:25%:5%, respectively.

Results: We first aimed at further optimizing and standardizing the gut organoid culture conditions. Isolated fibroblasts were characterized in terms of expression profiles of marker genes for different sub-populations that either support the stem cell compartment or the differentiation of the cells at the top of the villi. In parallel expression profiles of gut organoids alone or in co-culture with fibroblasts were performed and showed that both cell types profoundly influence each other. Optimized liver organoids were used as proof of principle in liver toxicity experiments where phtalates (DEHP) were applied and their hepatotoxic potential were investigated on the cellular level showing that only the 3D setup indeed simulates the in vivo situation.

Discussion/Conclusion: We successfully generated both, gut and liver organoids that can in the future be used to study interactions of both organs in a manner that will be much more translatable to the human in vivo situation than conventional setups.

10

Expression and function of neuroblastoma RAS viral oncogene homolog in hepatocellular carcinoma

Anne Gaza[1], Claus Hellerbrand[1,2], Anja Katrin Bosserhoff[1,2], Peter Dietrich[1]
[1]Institute for Biochemistry, Emil-Fischer-Zentrum, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany
[2]Comprehensive Cancer Center (CCC) Erlangen-EMN, Erlangen, Germany

Introduction: RAS proteins are amongst the most prominent oncogenes in many types of inflammation-associated cancers like colon- and pancreatic cancer. However, in hepatocellular carcinoma (HCC), the role of RAS proteins is mostly unexplored, likely because RAS genes are uncommonly mutated (< 4%) in HCC. Therefore, our aim was to focus on the function and prognostic value of Neuroblastoma RAS viral oncogene homolog (NRAS), which is largely unexpolored in HCC.

Methods: The HCC cell lines PLC, HepG2, Hep3B and isolated primary human hepatocytes (PHH) were used. Si-RNA-Pools were used for NRAS-Knockdown. Cell proliferation was analyzed using real-time cell proliferation assays. Clonogenicity assays and cell migration assays (Boyden chamber) were also performed. The Cancer Genome Atlas (TCGA), the Oncomine™ cancer microarray database and GEO datasets were analyzed to investigate the expression of NRAS in HCC and its effects on patient survival.

Results: NRAS mRNA levels were strongly overexpressed in human HCC cells as compared to hepatocytes. *In vivo*, HCC patient samples showed elevated NRAS expression levels in metastatic as compared to non-metastatic HCC. Moreover, two different murine HCC models showed enhanced NRAS expression in HCC development. Functionally, NRAS-Knockdown only slightly reduced proliferation and clonogenicity and had no effects on migration and PI3K-/AKT- and RAF-/ERK-signaling in HCC cell lines. Microarray and TCGA dataset analysis confirmed that NRAS expression was enhanced in HCC. Furthermore, high NRAS expression was identified to be a strong negative predictor of patient survival in HCC.

Conclusions: NRAS expression is upregulated in HCC development and progression *in vitro* and *in vivo* and high levels of NRAS predict poor patient outcome. However, NRAS inhibition revealed only slight, non-significant effects on proliferation and clonogenicity, pointing to potential intrinsic "rescue" mechanisms after NRAS-Knockdown. The current study supposes that NRAS expression could serve as a novel diagnostic and prognostic biomarker in HCC.

The role of IL-22 and IL-22BP in liver metastasis

Anastasios Giannou[1], Jan Kempski[1], Laura Garcia Perez[1], Ahmad Mustafa Shiri[1], Leonie Brockmann[1], Dimitra Zazara[3], Jöran Lücke[1], Lilan Zhao[2], Tanja Bedke[1], Theodora Agalioti[2], Nicola Gagliani[1,2], Samuel Huber[1]
[1]Sektion für Molekulare Immunologie und Gastroenterologie, I. Medizinische Klinik und Poliklinik, Universitätsklinikum Hamburg-Eppendorf, Germany
[2]Klinik und Poliklinik für Allgemein-, Viszeral- und Thoraxchirurgie, Universitätsklinikum Hamburg-Eppendorf, Germany
[3]Zentrum für Geburtshilfe, Kinder- und Jugendmedizin, Klinik und Poliklinik für Geburtshilfe und Pränatalmedizin, Universitätsklinikum Hamburg-Eppendorf, mburg, Germany

Introduction: Metastasis is a hallmark of malignancy and a common cause of cancer-related death (Hanahan D, Weinberg RA. 2011; Ferlay J, et al. 2015). It is known that approximately 80% of colorectal cancer patients develop liver metastasis. Every aspect of the metastatic process needs to be elucidated. The IL-22-IL-22BP system is an important novel target in cancer research. Although the role of IL-22 and IL-22BP in the development of colorectal cancer has been recently identified (Huber S, et al. 2012), its contribution to colon cancer metastasis remains unknown.

Methods: Liver metastasis models were used to study the metastatic pattern in WT, *IL22*$^{-/-}$ and *IL22bp*$^{-/-}$ mice. The mice were injected with tumor cells intrasplenically and then analysed for metastasis formation and immune cell infiltration. To investigate the migratory potential of intestinal IL-22 producing cells to the metastatic nische, we used Kaede transgenic mice, in which cells can be photo-converted and thus marked using UV light.

Results: Liver metastasis in mice was associated with an increased adaptive and innate immune response. Liver metastatic sites were infiltrated by CD11b^{+}Ly6C^{+}, CD11b^{+}Ly6G^{+} and T-cells (CD4^{+}, CD8^{+}) producing IL-17 and IL-22. Interestingly, the most abundant cell population in the metastatic sites were the CD8^{+} T-cells. Part of these cells was originating from the intestine, as seen by using the Kaede transgenic mice. Upon intra-splenic injection of colon cancer cells *IL22*$^{-/-}$ mice exhibited significantly reduced liver metastatic sites compared to WT and *IL22bp*$^{-/-}$ transgenic mice. The decreased tumor metastatic potential in *IL22*$^{-/-}$ mice was correlated with a reduced number of tumor circulating cells (TCC) in comparison to WT and *IL22bp*$^{-/-}$ mice. Furthermore, in the presence of IL-22 increased tumor cell extravasation was observed. In agreement to these findings, IL-22 was also upregulated in human metastasis samples.

Discussion/Conclusion: Our data highlight that liver metastasis is linked to an increased inflammatory response, which is partly mediated by the liver-gut axis. IL-22 appears to act as a key mediator in the liver metastatic process, by enhancing tumor cell extravasation and, therefore, could be a possible therapeutic target.

12

Reduction of carcinogenesis-associated pathways by pharmacological inhibition of the cannabinoid receptor 1 in the murine Abcb4$^{-/-}$ mouse model

Nora Helmrich, Yuri Churin, Annette Tschuschner, Martin Roderfeld, Elke Roeb
Department of Gastroenterology, Justus-Liebig University Giessen, Germany

Introduction: The endogenous endocannabinoid system is involved in controlling inflammatory processes in the liver. Previous animal studies showed a significant reduction of liver fibrosis and inflammation in chronic liver diseases through antagonising the endocannabinoid receptor 1 (CB1). The current study aimed to investigate the activation of the pre-carcinogenic JNK pathway in the Abcb4$^{-/-}$ mouse model and the effect of the CB1 antagonist rimonabant on these processes.

Methods: After weaning, male Abcb4$^{-/-}$ mice were treated with oral ACEA (CB1 receptor agonist) or rimonabant (CB1 receptor antagonist) until the age of 16 weeks. Liver, serum and abdominal fat were isolated and analysed by serum analysis, qRT-PCR, Western blot and immunohistochemistry. Abcb4$^{-/-}$ mice and Balb/C wild-type (WT) mice served as controls.

Results: By the pharmacological antagonisation of the CB1 receptor by rimonabant we demonstrated a reduction of both, the expression of mediators which are involved in the acute phase reaction (LCN2) and the activation of hepatocellular carcinoma (HCC) associated pathways (JNK, c-Jun, STAT3, cyclin D1) to a wild type level. The stimulation of the CB1 receptor enhanced the expression of acute phase proteins such as LCN2 and the activation of the proto-oncogenes JNK, c-Jun, cyclin D1 and STAT3.

Discussion/Conclusion: With the current study we could show that antagonising the CB1 receptor pathway led to a reduction of the acute phase response and a decreased activation of proto-oncogenes in the Abcb4$^{-/-}$ mouse model. Thus, the antagonisation of the CB1 receptor could be a new therapeutic concept for the treatment of cholestatic diseases and associated carcinogenesis.

Dietary cholesterol promotes transition from hepatic steatosis to steatohepatitis/NASH particularly in combination with a PUFA-rich Western-type diet

Janin Henkel[1], Manuela Kuna[1], Charles Dominic Coleman[1], Fabian Gellert[2], José Pedro Castro[3], Gerhard Paul Püschel[1]
[1]Department of Nutritional Biochemistry, Institute of Nutritional Science, University of Potsdam, Nuthetal, Germany
[2]Animal Facility, German Institute of Human Nutrition, Nuthetal, Germany
[3]Department of Molecular Toxicology, German Institute of Human Nutrition, Nuthetal, Germany

Introduction: Non-alcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH) are hepatic manifestations of the metabolic syndrome. Many animal models of NAFLD/NASH currently used lack clinical features of either NASH or metabolic syndrome, such as hepatic inflammation and fibrosis (e.g., high-fat diets) or overweight and insulin resistance (e.g., methionine-choline-deficient diets), or they are based on monogenetic defects (e.g., ob/ob mice). Dietary cholesterol is discussed to contribute to the transition from benign steatosis to the potentially fatal NASH.

Methods: In the current study, C57BL/6 mice were fed a chow diet (STD), a Western-type diet containing soybean oil with high n-6-PUFA (SOD) or a comparable high-fat diet mainly consisting of lard (LAD), supplemented either without or with 0.75% cholesterol (SOD+Cho, LAD+Cho) for 20 weeks.

Results: In contrast to SOD without cholesterol, both lard-based diets (LAD, LAD+Cho) and the soybean-oil diet with cholesterol (SOD+Cho) induced obesity and hepatic steatosis. However, only the SOD+Cho group in addition presented signs of inflammation and fibrosis. Although serum cholesterol levels were elevated in all 4 fatty diets, the hepatic cholesterol content was strongly increased solely in the SOD+Cho group whereas cholesterol levels were mildly elevated in the LAD+Cho group. Additionally, marker for oxidative stress were more pronounced in livers of mice fed a n-6-PUFA-rich SOD/SOD+Cho diet compared to the saturated fatty acid-rich LAD/LAD+Cho diets. Markers for immune cell infiltration, fibrosis, macrophage-recruiting chemokines and proinflammatory cytokines were solely increased in livers of SOD+Cho-fed mice resulting in a strong NASH phenotype. By contrast, in livers of LAD+Cho-fed mice markers for inflammation were only mildly increased while markers for fibrosis were not altered.

Discussion/Conclusion: Therefore, dietary cholesterol may be harmful to the liver, in particular when administered in combination with polyunsaturated fatty acids that favor lipid peroxidation.

14

Identification of the binding peptide sequence of cytokeratin 18 to the large hepatitis B surface protein

A. Huhn[1], Y. Churin[1], D. Glebe[2], T. Eckert[3,4], D. Schröder[1], M. Roderfeld[1], E. Roeb[1]
[1]Gastroenterology, Medical Clinic II, Justus-Liebig-University Giessen,
[2]Institute of Virology, Justus-Liebig-University Giessen,
[3]Institute of Biochemistry and Endocrinology, Faculty of Veterinary Medicine, Justus-Liebig-University Giessen,
[4]Department of Chemistry and Biology, University of Applied Sciences Fresenius, Idstein, Germany

Introduction: Cytokeratin 18 (CK18) is an intermediate filament protein expressed by monolayer epithelial cells such as hepatocytes. CK18 is involved in the processes of apoptosis, cell growth, protection against cytotoxic stress and positioning of the proteins and organelles in the cell. Cytokeratins interact with bacteria, viruses and parasites in the host cell. Some cytokeratins show a direct influence on infection, replication and release of pathogens from the host cells.
Hepatitis B surface proteins can cause severe liver damage with hepatocyte apoptosis, secondary inflammation processes and hepatocarcinogenisis (Chisari 1987, 1989). Accumulation and aggregation of the large hepatitis B surface protein (LHBs) in the endoplasmic reticulum leads to ER-stress and apoptosis of the involved hepatocytes. Our goals are to prove the interaction between CK18 and LHBs and to identify the LHBs-binding peptide sequence of CK18.

Methods: The interaction of CK18 and LHBs was analyzed in the LHBs-expressing hepatocyte cell line Huh7 by using co-immunoprecipitation. Four overlapping CK18 partial proteins were cloned in the expression vector c-Flag-pcDNA3 and transfected in the LHBs-expressing fibroblast cell line NIH3T3. The interaction of CK18-partial peptides and LHBs was analyzed with co-immunoprecipitation and Western Blot. The binding peptide sequence of CK18 with LHBs was analyzed via molecular dynamic simulation.

Results: Co-immunoprecipitation showed an interaction of CK18 and LHBs in cell culture. The LHBs binding peptide sequence of CK18 was identified between amino acid 87 and 192.

Discussion/Conclusion: By identifying the LHBs-binding peptide sequence of CK18 we can analyze the applicability for the treatment of hepatitis-B-infections. Our findings contribute to the apprehension of the pathophysiology of HBV-surface proteins.

References:

1. Chisari FV, Filippi P, Buras J, McLachlan A, Popper H, Pinkert CA, Palmiter RD, Brinster RL. Structural and pathological effects of synthesis of hepatitis B virus large envelope polypeptide in transgenic mice. Proc Natl Acad Sci USA. 1987;84:6909–13.

2. Chisari FV, Klopchin K, Moriyama T, Pasquinelli C, Dunsford HA, Sell S, Pinkert CA, Brinster RL, Palmiter RD. Molecular pathogenesis of hepatocellular carcinoma in hepatitis B virus transgenic mice. Cell. 1989;59:1145–56.

15

Hepatic CD36-dependent accumulation of lipids in HBs transgenic mice

Karuna Irungbam, Yuri Churin, Annette Tschuschner, Dagmar Leder,
Martin Roderfeld, Elke Roeb
Schwerpunkt Gastroenterologie, Justus-Liebig-Universität Giessen, Germany

Introduction: Chronic hepatitis B infection is a serious global health problem. Experimental investigations have suggested, that HBV infection is a potential trigger of liver steatosis. Fatty acid translocase (FAT/CD36) is an 88 kDa single chain membrane glycoprotein expressed in many tissues and cell types. CD36 is a multi-ligand class B scavenger receptor with high affinity for lipids and lipid-containing ligands. However, the role of CD36 in hepatic lipid metabolism is still not well understood. The aim of the present study is to define the role of CD36 in hepatic lipid metabolism and development of steatosis.

Methods: WT, HBs (HBV transgenic mice), IRF3/7 DKO (double knock out) and $IRF3^{-/-}/7^{-/-}/HBs^{+/-}$ (triple transgenic mice) mice group were used for the study. Steatosis was assessed histologically, thin layer chromatography (TLC) and quantitatively by triglycerides assay kit. qPCR method was used for studying the genes involved in lipogenic pathways. qPCR and western blot analysis were used to assess hepatic FAT/CD36 expression. Immunostaining and Western blot analysis were used to determined subcellular distribution of CD36.

Results: We have observed that HBs (HBV transgenic), IRF3/7 DKO (double knock out) and $IRF3^{-/-}/7^{-/-}/HBs^{+/-}$ (triple transgenic) mice developed progressive steatosis and inflammation in comparison to wild type control (WT). Ppar, Cyp4a14, Fabp4, Fabp1, Cd36, Mgat1, and Adrp expression levels were increased in HBs and $IRF3^{-/-}/7^{-/-}/HBs^{+/-}$ transgenic mice in comparison to WT. Immunostaining and Western blot analysis revealed increased localization of CD36 at the hepatocytes plasma membrane of HBs transgenic mice as compared to IRF DKO or WT control. Similarly the rate limiting enzymes involved in TG synthesis, MGAT-1 was also found to be upregulated. The upregulation of ADRP (adipose differentiation-related protein) also promotes TG accumulation by binding to lipid droplets and protection from lipase activity.

Discussion/Conclusion: Thus, increased lipid accumulation in the liver of HBs transgenic mice could result from increased FAT/CD36 localization at the hepatocytes plasma membrane and might be modulated by type I interferon signaling.

Beneficial effects of vitamin D treatment in an obese mouse model of non-alcoholic steatohepatitis

D. Jahn[1], D. Dorbath[1], S. Kircher[2], H.M. Hermanns[1], A. Geier[1]
[1]Division of Hepatology, University Hospital Würzburg, Germany
[2]Institute of Pathology, University of Würzburg, Germany

Introduction: Serum 25-OH vitamin D3 (VD3) levels negatively correlate with obesity and the severity of non-alcoholic steatohepatitis (NASH) in humans. However, the mechanisms linking disease progression to low VD3 status are not entirely clear. In this study, we tested the hypothesis that VD3 treatment ameliorates NASH in diet-induced obese mice and analysed whether this is associated with VD3-dependent changes of intestinal physiology.

Methods: To induce obesity and NASH, C57BL/6J mice were fed a high-fat/high-sugar diet (HFSD) with low amounts of VD3 for 16 weeks. The effects of preventive and interventional VD3 therapy were studied by treatment with high amounts of dietary VD3 starting at week 1 (preventive) or week 12 (interventional), respectively. Analyses were carried out on the level of liver histology as well as hepatic and intestinal gene expression.

Results: Animals receiving HFSD with low VD3 developed obesity, histologically-defined NASH and liver fibrosis after 16 weeks. This phenotype was associated with increased gene expression levels of lipogenic, inflammatory and pro-fibrotic markers in the liver and with increased expression of pro-inflammatory genes in the intestine. Interestingly, preventive but not interventional VD3 treatment resulted in improvements of liver histology, including a significant decrease of steatosis and a trend towards a lower NAFLD activity score. These hepatic effects were associated with a reduction of the expression of inflammatory markers in the gut of VD3-treated animals.

Discussion/Conclusion: This study reveals a beneficial impact of VD3 treatment on disease progression in an obese NASH mouse model. Importantly, our observations suggest that timely initiation of VD3 supplementation (preventive vs. interventional) is critical for treatment outcome and argue for an important role of the gut to mediate the therapeutic effects of VD3.

17

Pilot single-center study of measurement of liver stiffness of patients with ulcerative colitis and Crohn's disease on long-term therapy with thiopurines

G. Karampekos, G. Fillipidis, N. Viazis, G. Mantzaris
Gastroenterology Clinic, Evangelismos General Hospital, Athens, Greece

Introduction: Thiopurines are widely used for steroid-dependent Crohn's disease (CD) or ulcerative colitis (UC). Long-term treatment in male patients, who have surgically removed more than 50 cm of their terminal ileus for CD, may lead to nodular focal hyperplasia and portal hypertension, while singular cases of hepatic fibrosis have been reported. This study aims to investigate whether the long term use of thiopurines can lead to hepatic fibrosis in patients with UC or CD.

Methods: We included patients with UC or CD treated with AZA/6MP for ≥ 3 years, without previous hepatic disease, bile stones, alcohol consumption or biliary surgery and with > 90% successful measurements of liver stiffness (PKa). The patients' demographic and disease characteristics, BMI, previous surgeries and substance addictions were recorded, and then they underwent upper abdominal ultrasound followed by fibroscan. The mean was calculated as the median of at least 10 measurements per session. We considered normal measurements those with PKa < 7.0.

Results: 22 patients were examined, with median age 38.5 (22–64) years, 19 with CD, none of whom were drinkers, 11 smokers. The median duration of treatment with AZA was 6.4 (4–11) years. The median of BMI was 23.94 (20.2–28.6) kg/m^2. Three patients had non-alcoholic fatty liver disease (NAFLD), without any abnormal liver function tests (LFT). Six patients with CD were submitted to right-sided hemicolectomy either before (n = 3) or after the fibroscan. The fibroscan was repeated after 1 year for 14 patients and after 2 years for 1 patient, without any difference in results. None presented abnormal LFTs. Nevertheless, 2 patients with CD, NAFLD and BMI > 27 had borderline values of PKa measured twice with a year's distance between measurements. (7.4/7.6 and 7.6/7.5).

Discussion/Conclusion: This pilot study shows that long term therapy with thiopurines does not cause hepatic fibrosis. Special care must be given to patients with NAFLD and increased BMI.

Prospective, longitudinal cohort study of the pathologic increase in liver stiffness: Early diagnosis of liver disease in cystic fibrosis

Victoria Klotter[1], Caroline Gunchick[1], Enno Siemers[1], Helge Hudel[2], Martin Roderfeld[1], Elke Roeb[1]
[1]Department of Internal Medicine, Division of Gastroenterology, Justus-Liebig-University Giessen, Germany
[2]Institute for Medical Informatics, Justus-Liebig-University Giessen, Germany

Introduction: About 30% of patients with cystic fibrosis (CF) develop CF-associated liver disease (CFLD). Recent studies have shown that transient elastography (TE), a method to quantify liver stiffness, allows non-invasive diagnosis of CFLD in adults and children with CF. Within this 5-year follow-up study, we aimed to prospectively identify patients at risk for development of fibrosis and CFLD by longitudinal analysis of liver stiffness and fibrosis scores.

Methods: 16 adult and 36 pediatric CF patients were examined by transient elastography for 4–5 years.
All patients showed an initial liver stiffness below < 6.3 kPa, which is an optimal threshold value indicative of CFLD calculated by our research group based on ROC analysis [1]. TE, APRI-, and FIB-4-scores were assessed and compared by Kruskal-Wallis test and analysed by receiver operating characteristic (ROC) analysis. Frequencies were compared by X^2-test.

Results: Among the 36 pediatric patients participating in this study, a subgroup of 9 patients developed liver stiffness > 6.3 kPa after 4–5 years. To identify those patients with a continuous rise of liver stiffness and a final stiffness above the threshold of ≥ 6.3 kPa, the ROC analysis was used to calculate the optimal cut-off for the mean increase of $\Delta TE_{cut\text{-}off}$ 0.38 kPa/a (the subgroup with increasing liver stiffness was labelled TE_{inc}). APRI- and FIB-4 scores confirmed the rationale for grouping. The frequency of CFLD assessed by conventional diagnosis was significantly higher in TE_{inc} group compared to the control group (TE_{norm}). None of the adult CF patients matched criteria for TE_{inc} group.

Discussion/Conclusion: For the first time, it was shown that the non-invasive and reproducible longitudinal assessment of transient elastography allows identification of juvenile patients before reaching the level of stiffness indicating established CFLD (6.3 kPa). According to this, the cut-off for pathologic increase of liver stiffness of $\Delta TE_{cut\text{-}off}$ 0.38 kPa/a is recommended.

Reference:

1. Rath et al. PLoS One. 8(3) 2013.

19

Effects of combined low-dose spironolactone plus vitamin E vs. vitamin E monotherapy on insulin resistance, non-invasive indices of steatosis and fibrosis, and adipokine levels in non-alcoholic fatty liver disease: A randomized controlled trial

S.A. Polyzos[1], J. Kountouras[1], C.S. Mantzoros[2], V. Polymerou[3], P. Katsinelos[1], M. Doulberis[4]
[1]Department of Medicine, Second Medical Clinic, Aristotle University of Thessaloniki, Ippokration Hospital, Thessaloniki, Greece
[2]Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA
[3]Department of Biopathology, Biomedicine Laboratories, Athens, Greece
[4]Department of Internal Medicine, Bürgerspital Hospital of Solothurn, Switzerland

Background/Methods: The beneficial effects of mineralocorticoid receptor blockade by spironolactone have been shown in animal models of non-alcoholic fatty liver disease (NAFLD). The aim of the present 52-week randomized controlled trial was to compare the effects of low-dose spironolactone and vitamin E combination with those of vitamin E monotherapy on insulin resistance, non-invasive indices of hepatic steatosis and fibrosis, liver function tests, circulating adipokines and hormones in patients with histologically confirmed NAFLD. Thirty-one (23 women) participants with NAFLD (15 with simple steatosis and 16 with non-alcoholic steatohepatitis [NASH]) were randomly assigned to group1 (n = 17; 11 women) or group 2 (n = 14; 12 women). Homeostasis model of assessment of insulin resistance (HOMA-IR) and non-invasive indices of steatosis and fibrosis were calculated. Analysis was intention-to-treat.

Results: NAFLD liver fat score, an index of steatosis, decreased significantly in the combination treatment group ($p = 0.028$), but not in the vitamin E group, and the difference for group*time interaction was significant ($p = 0.047$). Alanine amino-transferase-to-platelet ratio index, an index of fibrosis, did not change. Insulin levels and HOMA-IR decreased significantly only within the combination group ($p = 0.011$ and $p = 0.011$, respectively). Vaspin levels decreased in group 2 ($p = 0.02$), but not in group 1, but difference in group*time interaction was not significant. Resistin showed a non-significant trend within groups ($p = 0.06$), but per-group analysis did not reveal significant differences (within group 1: $p = 0.09$; within group 2: $p = 0.15$). No significant differences were observed in other adipokines. Group*time interaction did not change after adjustment for changes in waist circumference.

Discussion/Conclusion: In conclusion, the combined low-dose spironolactone plus vitamin E regimen significantly decreased NAFLD liver fat score. Larger-scale trials are needed to clarify the effect of low-dose spironolactone on hepatic histology.

Serum sterol levels indicate distorted cholesterol homeostasis in cirrhotic patients with primary biliary cholangitis

Marcin Krawczyk[1,2], Ewa Wunsch[3], Dieter Lütjohann[4], Frank Lammert[1], Piotr Milkiewicz[3,5]
[1]Department of Medicine II, Saarland University Medical Center, Homburg, Germany
[2]Laboratory of Metabolic Liver Diseases, Centre for Preclinical Research, Department of General, Transplant and Liver Surgery, Medical University of Warsaw, Poland
[3]Translational Medicine Group, Pomeranian Medical University, Szczecin, Poland
[4]Institute for Clinical Chemistry and Clinical Pharmacology, University of Bonn, Germany
[5]Liver and Internal Medicine Unit, Department of General, Transplant and Liver Surgery, Medical University of Warsaw, Poland

Introduction: In humans new cholesterol derives from *de novo* synthesis and intestinal absorption. Serum cholesterol precursor (e.g., lathosterol, desmosterol) and plant sterol levels (e.g., sitosterol, campesterol) represent valid surrogate marker for cholesterol biosynthesis and intestinal absorption, respectively. Since chronic liver diseases modulate cholesterol homeostasis, we systematically investigated sterol serum levels in patients with primary biliary cholangitis (PBC) with and without liver cirrhosis.

Methods: Overall, we recruited 111 non-transplanted PBC patients (age 22–83 years, 101 females). In this cohort, a total of 30 individuals presented with liver cirrhosis at diagnosis. Serum concentrations of plant sterols, cholesterol and its precursors were measured by gas chromatography/mass spectrometry (GC/MS). Individuals with results suggesting familial hypercholesterolemia or phytosterolemia were excluded. Serum sterols were compared between cirrhotic and non-cirrhotic patients using non-parametric tests.

Results: PBC patients with liver cirrhosis demonstrated significantly higher serum sitosterol and campesterol concentrations than non-cirrhotic individuals ($p = 0.0002$ and $p = 0.007$, respectively). Serum levels of lathosterol and desmosterol were lower in these patients ($p = 0.0001$ and $p = 0.01$, respectively), who also displayed a trend to lower serum cholesterol ($p = 0.06$). In cirrhotic patients we identified increased sitosterol:cholesterol and campesterol:cholesterol but decreased lathosterol:cholesterol ratios (all $p < 0.0001$). Overall, the ratios of phytosterols to cholesterol precursors were significantly (all $p > 0.0001$) increased in patients with liver cirrhosis as compared to non-cirrhotic patients.

Discussion/Conclusion: PBC patients with liver cirrhosis are characterized by decreased cholesterol synthesis and increased sterol absorption as compared to non-cirrhotic individuals. The determination of serum sterols may improve the clinical assessment and stratification of patients with PBC.

21

The intestinal microbiota of patients with PSC are different from healthy controls and patients with ulcerative colitis across geographical regions

T. Liwinski[1], F. Heinsen-Groth[2], M. Rühlemann[2], R. Zenouzi[1], M. Kummen[3], J. Hov[3], T. Karlsen[3], C. Bang[2], A. Lohse[1], A. Franke[2], C. Schramm[1]
[1]I. Department of Medicine, University Medical Center Hamburg-Eppendorf, Germany
[2]Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Germany
[3]Norwegian PSC Research Center, University Hospital Rikshospitalet, Oslo, Norway

Introduction: Primary sclerosing cholangitis (PSC) is a cholestatic liver disease which is highly associated to inflammatory bowel disease (IBD). Single-centre studies found changes in the faecal microbiome of patients with PSC. However, it is unknown whether PSC has a common microbiome-signature across geographical regions.

Methods: Stool samples were collected from two independent cohorts from Norway and Germany. The pooled patient population included 388 patients, 137 patients with PSC (75 with concurrent IBD), 88 patients with ulcerative colitis (UC) and 133 healthy controls (HC). The microbiomes were profiled by 16S-ribosomal-RNA-gene sequencing targeting the V1–V2 region, biodiversity-analysis, machine-learning classification and receiver operating characteristic (ROC) analysis.

Results: On the regional level as well as in the pooled cohort, the beta-diversity was different between PSC and HC ($p < 0.001$) as well as between PSC and UC ($p < 0.001$). In each test, the disease state had the strongest impact on microbial beta-diversity after adjusting for age, gender, BMI and smoking-status. No differences in beta-diversity were observed between PSC with or without colitis. We tried to assess, whether the faecal microbial profile can be used to differentiate between healthy and diseased individuals. A random forest classifier trained on the German cohort yielded an area under the curve (AUC) of 0.81 classifying the Norwegian population in PSC versus HC. Additionally, we trained random forests on stratified randomly split samples from the whole population including Norwegian and German patients. The classifiers achieved an AUC = 0.87 classifying between PSC versus HC and an AUC = 0.82 classifying PSC versus UC in the respective validation samples.

Discussion/Conclusion: The faecal microbiota of patients with PSC are distinct from both HC and UC and allow an accurate classification across different geographical areas. The microbiome is similar between PSC patients with or without colitis, strongly suggesting that the liver disease drives the observed differences.

Combined effects of curcumin and xanthohumol in *in vitro* models of hepatic steatosis and fibrosis

Abdo Mahli, Tatjana Seitz, Ali Chiet, Claus Hellerbrand
Institute of Biochemistry (Emil-Fischer Zentrum), Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany

Introduction: Xanthohumol (XN) is the principal prenylated chalconoid of the female inflorescences of the hop plant. XN has shown various beneficial effects including anti-inflammatory, antioxidant, hypoglycemic activities, and anticancer effects. Curcumin (CR) is the most active flavonoid of turmeric (*Curcuma longa*) and it has been used as hepatoprotective agent in acute and chronic liver injury models.

The aim of this study was to analyze the combined effects of XN and CR on fatty acids-induced hepatic steatosis as well as on activated hepatic stellate cells in *in vitro* models.

Methods and results: Human HepG2 hepatoma cells were pre-incubated with serial concentrations of XN (up to 10 µM), CR (up to 10 µM) or in combinations for 1 h. Subsequently, cells were exposed to 0.4 mM fatty acids (oleate) for 24 h. Under these conditions, neither XN nor CR or their combinations have shown any toxic effects on hepatocytes as indicated by levels of transaminases in the supernatant and microscopic documentation. Oleate treatment has significantly induced the hepatocellular triglyceride accumulation and the gene expression of pro-inflammatory cytokines (IL-8 and ICAM-1). These oleate-induced effects were slightly but significantly inhibited by XN or CR pre-incubation in dose-dependent manner. Combination of XN and CR treatment has additively (at low concentrations) or synergistically (at higher concentrations) further reduced the hepatocellular triglyceride content as well as the pro-inflammatory gene expressions. Next, we analyzed the combined effects of XN and CR on activated hepatic stellate cells, the central mediator of hepatic fibrosis. Also in these cells, XN and CR synergistically reduced the gene expression of pro-fibrogenic factors (Coll-I and TGFβ1) as well as the pro-inflammatory factors (MCP1 and IL-8) in a dose dependent manner.

Conclusion: XN and CR exhibit synergistic beneficial effects in *in vitro* models of fatty acids-induced lipid accumulation and inflammation in hepatoma cells and pro-fibrogenic and pro-inflammatory gene expression of hepatic stellate cells. Together with previous studies showing the safety of XN or CR applications in men our data suggest XN/CR-combination as promising therapeutic strategy for the prevention and treatment of (non-alcoholic) fatty liver disease.

23

Inhibition of gut microbiota can be a therapeutic target to reduce liver fibrosis

Fatma El Zahraa Ammar Mohamed[1,2#], Steven Dooley[1#], Nathan A. Davies[4], Seddik Hammad[1,3], Abeba Habtesion[4], Rajiv Jalan[4]
[1]Molecular Hepatology Section, Department of Medicine II, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany
[2]Department of Pathology, Faculty of Medicine, Minia University, Minia, Egypt
[3]Department of Forensic Medicine and Veterinary Toxicology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt
[4]UCL Institute for Liver and Digestive Health, Royal Free Hospital, London, UK
[#]Equal contribution
E-Mail: fatma.mohamed@medma.uni-heidelberg.de

Introduction: Despite of their beneficial effects, alterations of gut microbiota in liver diseases enhances endotoxins production. In addition, increased gut permeability in this setting maintains continuous liver exposure to gut-produced endotoxins, which in turn leads to persistent liver inflammation and fibrosis.

Aim of the work: Study the effect of reducing the endotoxin level – using the well-known anti-bacterial agent norfloxacin – on liver disease in a rat model of liver cirrhosis.

Methods: Eighteen Fisher rats divided into 3 groups; 1st treated with diethyl-nitrosamine (DEN) and oral N-nitrosomorpholine (NMOR). 2nd was treated with the same treatment + norfloxacin (20 mg/kg/day) from the beginning of the experiment to 14 weeks (end of experiment). 3rd group was served as a control. Endotoxin assay and liver functions were performed on animals' blood. H&E and reticulin were performed on liver tissue and TNF-α were measured.

Results: There was a significant reduction of endotoxin level ($p < 0.02$) and liver transaminases, namely ALT ($p < 0.05$) and AST ($p < 0.01$) in the norfloxacin treated group comparing to the DEN and NMOR group alone. With reticulin staining there was significantly reduction in liver fibrosis in the group treated with norfloxacin (score: 1 [0–2]) compared with the DEN and NMOR group (score: 4 [3–5]) ($p < 0.03$). There was also reduction of TNF-α level with norfloxacin treatment.

Conclusion: The results of this study suggest that selective decontamination of the gut by using norfloxacin leads to decrease circulating endotoxin may be a novel therapeutic strategy to treat fibrosis and enhance liver function.

Schistosoma mansoni activates protooncogene c-Jun in hamster model

Sevinc Padem[1], Christoph G. Grevelding[2], Thomas Quack[2], Yuri Churin[1], Annette Tschuschner[1], Martin Roderfeld[1], Elke Roeb[1]
[1]Department for Gastroenterology, [2]Department for Parasitology, Justus-Liebig-University Giessen, Germany

Introduction: During an infection with the parasitic flatworm *S. mansoni* the schistosomal eggs of paired adult worms can be lodged in the walls of small portal veins, where they induce inflammatory reactions, leading to multiple granulomas in the liver. Clinical studies and animal experiments suggest that schistosomiasis acts as a cofactor in case of hepatitis B or C coinfection for the development of hepatocellular carcinoma (HCC). The aim of the following study is to examine whether there is any activation of protooncogenes, which are essential for the development of HCC.

Methods: To analyse expression, localisation and activation of c-Jun in the livers of *S. mansoni* (♀+♂) infected hamsters, immunohistochemistry and Western blot analyses were performed. Monosexual infected hamsters served as controls.

Results: In hepatocytes of bisexual infected hamsters, nuclear translocation of c-Jun was detected around the granulomas, whereby a considerable activation in direction to the central vein following the blood flow could be observed. The activation of c-Jun was also found around freshly settled eggs without granulomatous inflammatory changes. Using Western blot and densitometric analysis it has been shown that there was a significantly higher hepatic c-Jun expression and activation in the bisexual infected hamsters.

Conclusion: The activation of c-Jun in hepatocytes around freshly settled *S. mansoni* eggs and around granulomas in the direction of the blood flow suggests that the egg secreted factors could cause a permanent activation of the protooncogene c-Jun. The identification of a molecular biological connection between the soluble components of the eggs and the sustained activation of protooncogenes could help to elicit more information about the aetiology of HCC in the presence of coinfection with *S. mansoni*. Based on these findings new therapeutic targets to treat schistosomiasis and regarding complications like HCC could be detected.

25

Microbiota-dependent effects of IL-22

Penelope Pelczar, Morsal Said, Marius Böttcher, Samuel Huber
I. Medizinische Klinik und Poliklinik, Universitätsklinikum Hamburg-Eppendorf, Germany

Introduction: Interleukin-22 (IL-22) plays a protective role in tissue regeneration, it promotes mucosal healing and defense against pathogens in the intestine and liver. *IL-22*$^{-/-}$ mice were shown to exhibit an increased disease severity in the DSS-colitis model when compared to wild type mice. However, this observation is not the case in *IL-22*$^{-/-}$ mice bred in our animal facility in Hamburg. On the basis of these data we hypothesized that the specific composition of the intestinal microbiota might impact the role of IL-22 in intestinal and liver inflammation.

Methods: Fecal transplantation of two different microbiota compositions, Mb1 and Mb2, was used to alter the indigenous intestinal microbiota of WT, and *IL-22*$^{-/-}$ mice. Liver and intestine were analysed in steady state or upon challenge with DSS. Stool samples were also taken for microbiota analysis.

Results: Mouse stool screening from 2 different facilities (Mb1 and Mb2) confirms that there is a difference in the microbiota compositions. Change in *IL-22* expression was observed in the colon but not in the liver of these microbiota transplanted animals. The Mb2 composition resulted in increased intestinal inflammation when compared to the Mb1 composition, in both steady state and diseased conditions, suggesting that the Mb2 composition was more colitogenic. In contrast, the Mb2 microbiota seemed to have protective effects in the liver in a mouse model for Primary sclerosing cholangitis. Finally, *IL-22*$^{-/-}$ mice were more susceptible for colitis development in the presence of the Mb2, but not Mb1 microbiota, indicating that IL-22 might have flora-dependent tissue protective effect.

Discussion/Conclusion: Our data show that the intestinal microbiota impacts intestinal and liver disease. However, while one intestinal microbiota might be pathogenic in the intestine, it might be protective in the liver. Furthermore IL-22 seems to have flora dependent tissue protective effects.

Oral probiotic administration influences pro- and anti-inflammatory cytokines in NAFLD

O.M. Plehutsa, I.R. Sydorchuk*, A.R. Sydorchuk, L.P. Sydorchuk, O.Y. Khomko, I.I. Sydorchuk, R.I. Sydorchuk, O.B. Rusak
Bukovinian State Medical University, Chernivtsi, Ukraine
*Goethe University Frankfurt, Frankfurt, Germany

Introduction: Non-alcoholic fatty liver disease (NAFLD) is multi-factorial and insufficiently understood disease, frequently believed to be a part of metabolic syndrome with globally rising incidence. The role of pro- and anti-inflammatory cytokines in pathogenesis of NAFLD is generally well established. However, influence of diet and dietary interventions including probiotics is still unclear. We hypothesized that different probiotic compositions may have different impact not only on intestinal microbiota but also significant influence on immunity and inflammation in NAFLD.

Methods: Sixty patients with verified NAFLD participated in the study (42 men and 18 women, mean age 57.9 ± 4.8 years). All patients were randomized into the following groups: 1st group included all patients before treatment, 2nd group – 23 patients after standard unmodified treatment (EASL/EASD/EASO, 2016), 3rd group – 17 patients after additional treatment with oral probiotics (*Bifidobacterium longum* and *Enterococcus faecium*), 4th group – 20 patients after additional treatment with oral probiotics (*Bacillus subtilis* and *Bacillus licheniformis*). Control group included 17 practically healthy subjects. For research purpose pro- and anti-inflammatory cytokines transforming growth factor beta (TGF-β), tumor necrosis factor alpha (TNF-α) and IL-1β were typified (ELISA) before and after 60 days of treatment in all groups.

Results: IL-1β and TNF-α levels before treatment were almost twice higher than in practically healthy subjects; TGF-β level was 2.8 times higher than in control.

Cytokines	Cytokines concentration (pg/ml)				
	Study groups				Control (n = 17)
	1st (n = 60)	2nd (n = 23)	3rd (n = 17)	4th (n = 20)	
IL-1β	92.1 ± 2.8^{p}	70.9 ± 3.5^{p,p_1}	65.6 ± 4.2^{p,p_1}	72.2 ± 3.7^{p,p_1}	50.7 ± 3.6
TNF-α	86.1 ± 2.2^{p}	71.7 ± 3.5^{p,p_1}	83.3 ± 3.5^{p,p_2}	93.7 ± 4.2^{p,p_2}	46.3 ± 4.3
TGF-β	150.4 ± 5.0^{p}	142.1 ± 7.4^{p}	72.0 ± 3.9^{p,p_1,p_2}	63.5 ± 4.1^{p_1,p_2}	53.1 ± 3.6

Key: ^{p} – $p < 0.001$ compared to the data of the control; ^{p_1} – $p < 0.05$ compared to the data of the 1st group; ^{p_2} – $p < 0.05$ compared to the data of the 2nd group.

In 2nd group IL-1β and TNF-α levels decreased 22.9% and 16.8% accordingly, with still higher values compared to control group's results. TGF-β level did not statistically differ in the 2nd group after standard treatment. Probiotic administration in 3rd group caused IL-1β level decrease by 28.7%, TGF-β level decrease 2.1-fold with regard to 1st group, but all data were still higher than in control group – 29.4%, 35.6%, accordingly, especially TNF-α level – 79,9%. Probiotic administration in 4th group caused IL-1β

level decreasing by 21.6%, TGF-β level decreased 2.4 times and did not differ reliably from the control group; TNF-α level became certainly higher, comparatively to second and control groups.

Discussion/Conclusion: Standard therapy of NAFLD patients caused significant IL-1β and TNF-α levels concentration decrease without certain influence on TGF-β level. Various oral probiotic administration led to statistical IL-1β and TGF-β levels decrease, but did not significantly change TNF-α concentration. The idea of other pre- and probiotic compositions study in NAFLD looks promising.

12/15-Lipoxygenase-deficient mice show exacerbated experimental colitis and alterations in epithelial proliferation

Mohab Ragab[1,§], Annika Sünderhauf[1,§], Kilian von Medem[1], Florian Bär[1], René Pagel[2], Axel Künstner[3], Christian Sadik[4], Stefanie Derer[1], Christian Sina[1]
[1]Institute for Nutritional Medicine, University of Lübeck, Germany; [2]Institute of Anatomy, University of Lübeck, Germany; [3]Lübeck Institute of Experimental Dermatology, University of Lübeck, Germany; [4]Department of Dermatology, University of Lübeck, Germany
§ These authors contributed equally to this study.

Introduction: Inflammatory bowel disease (IBD), such as ulcerative colitis (UC) and Crohn's disease (CD) is characterized by chronic relapsing intestinal inflammation. However, the exact mechanism behind it still remains to be resolved. Arachidonate 15-lipoxygenase-1 (Alox15) is the key enzyme in the biosynthesis of lipid mediators (*e.g.*, lipoxins and resolvins) playing an important role in the alleviation of inflammation. Hence, the aim of the present study is to investigate the impact of Alox15 on intestinal barrier function within the context of experimental colitis.

Methods: Basal gene expression in colon samples of 12/15-LOX deficient mouse strain (*Alox15*$^{-/-}$) and wild-type C57BL/6 mice was determined by microarray analysis. Transcripts found to be differentially expressed were confirmed via reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) and Western blot analysis. Microbiome composition was analyzed via 16srRNA next generation sequencing. Finally, *Alox15*$^{-/-}$ as well as C57BL/6 mice were treated with 4% (w/v) dextran sodium sulfate (DSS) in the drinking water for 5 days followed by 3 days of normal drinking water.

Results: A genome wide expression analysis revealed a significant downregulation of Ki-67, a marker for cell proliferation, in *Alox15*$^{-/-}$ mice. This finding was confirmed at expression level via RT-qPCR and on protein level via Western blot. The microbiota analysis showed lower levels of *Firmicutes* and higher levels of *Bacteriodetes* in *Alox15*$^{-/-}$ mice than in control mice. Furthermore, depletion in *Akkermansia muciniphila* was observed in *Alox15*$^{-/-}$ mice. *Alox15*$^{-/-}$ mice exhibited a severe form of colitis as compared to control mice. The modified disease activity index resulted in higher scores for *Alox15*$^{-/-}$ than wild-type mice. In addition, histological analysis of colon samples showed higher grades of inflammation for *Alox15*$^{-/-}$ in comparison to control mice.

Discussion/Conclusion: Collectively, our data suggest a new potentially relevant mechanism linking Alox15 deficiency to aggravated experimental colitis through impairment of epithelial proliferation and alteration of microbiota composition.

28

IL-13 knockout reduces the activation of ER stress associated pathways in Abcb4$^{-/-}$ mice

Martin Roderfeld, Luisa Hahn, Annette Tschuschner, Elke Roeb
Gastroenterology, Medical Clinic II, Justus-Liebig-University Giessen, Giessen, Germany

Introduction: Recently, we demonstrated the phosphorylation of *Protein Kinase RNA-like Endoplasmic Reticulum Kinase* (PERK) and *Eukaryotic Translation Initiation Factor 2α* (eIF2α) indicating ER stress and unfolded protein response (UPR) in Abcb4$^{-/-}$ mice[1]. Furthermore, it has been shown that IL-13 may induce endoplasmic reticulum (ER) stress in the context of asthma [2]. With the current study we aimed to analyze the effect of IL-13 knockout on ER stress and UPR in the Abcb4$^{-/-}$ mouse model.

Methods: We generated Abcb4$^{-/-}$ and IL-13$^{-/-}$ double-knockout mice on fibrosis susceptible genetic background BALB/c. Molecular and cellular mechanisms of hepatic ER stress and UPR were investigated by Western blot and immunostaining in female mice at the age of 8 weeks.

Results: ER stress associated PERK and eIF2α were strongly phosphorylated in Abcb4$^{-/-}$ mice while this phosphorylation pattern was reduced in Abcb4$^{-/-}$IL-13$^{-/-}$ mice. Net amount of BIP/GRP78 expression was not altered significantly.

Discussion/Conclusion: The reduced activation of ER stress markers was clearly associated with ameliorated cholestasis. Nevertheless, it has to be proved whether reduction in ER stress improved cholestasis or vice versa. Our study may indicate that the modulation of IL-13 could be an important therapeutic candidate for the treatment of cholestasis associated ER stress.

References:

1. Zahner D, et al. Hepatitis B virus surface proteins accelerate cholestatic injury and tumor progression in Abcb4-knockout mice. Oncotarget. 2017;8:52560–70.

2. Wang X, et al. Lyn kinase represses mucus hypersecretion by regulating IL-13-induced endoplasmic reticulum stress in asthma. EBioMedicine. 2017;15: 137–149.

Optimisation of liver cell quantification in 3D scaffolds

Marc Ruoß[1], Carl Grom-Baumgarten[1], Leon Olde Damink[2], Serene M.L. Lee[3], Andreas K. Nüssler[1]
[1]Siegfried Weller Institute for Trauma Research, BG Trauma Center, Eberhard-Karls-University Tübingen, Germany
[2]Matricel GmbH, Herzogenrath, Germany
[3]Hepacult GmbH, Munich, Germany

Introduction: Primary human hepatocytes (PHH) are recognized to be the gold standard for metabolism and toxicity studies. However, PHHs have limitations, such as low availability and rapid de-differentiation in culture. Recent studies showed that cells which are cultured in 3D systems maintain their differentiated state and metabolic profile much longer compared to cells in 2D cultures. However, 3D culture techniques have an important drawback as cells cannot be easily detached from scaffolds. This makes it challenging to apply conventional normalisation strategies used in 2D cultures, such as quantitation of numbers or protein contents of detached cells. Thus, this study aimed to establish a practical normalisation method.

Methods: Different numbers of HepG2 cells and PHHs were plated onto self-made HEMA-based scaffolds, Collagen-Optimaix scaffolds (Matricel) and conventional plates for 2D culture. Different methods for cell quantification like resorufin production, LDH activity, and two different methods of DNA quantification (Cyquant, HOECHST) were tested to compare the cell recovery and linearity in 2D and 3D cultures.

Results: All tested methods were able to quantify the cell numbers in 2D and 3D cultures under certain conditions. However, there were considerable differences in sensitivity and reliability. The measurements of resorufin and LDH activity can only be used to a limited extent for normalisation in the toxicity tests and the HOECHST measurement showed limits in sensitivity. DNA quantification through usage of the Cyquant-Kit was very sensitive but not applicable if the scaffolds were cultured in phenol red containing medium before.

Discussion/Conclusion: The quantitative method which can be used for normalisation of a 3D scaffold culture depends not only on the type of the scaffold. The medium used, the cell type as well as the necessary sensitivity of the assay are important factors which must be considered before deciding on a method.

30

Effects of short-term dietary changes on immune system and beyond

Nicola Schaltenberg[1,2,3], Laura Fromann[2,3], Pasquale Scognamilio[3], Theodora Agalioti[2,3], Alexander Fischer[1], Penelope Pelzcar[2], Anna Worthmann[1], Ramez Wahib[3], Markus Heine[1], Ludger Scheja[1], Samuel Huber[2], Joerg Heeren[1], Nicola Gagliani[2,3,4]

[1]Department of Biochemistry and Molecular Cell Biology, University Medical Center Hamburg-Eppendorf; [2]I. Department of Medicine, University Medical Center Hamburg-Eppendorf; [3]Department of General, Visceral and Thoracic Surgery, University Medical Center Hamburg-Eppendorf, Germany; [4]Department of Medicine Solna, Karolinska Institute, Solna, Sweden

Introduction: In western countries, both obese and conscious eaters regularly expose themselves to unhealthy diets, e.g. at Christmas. Short exposure to fatty diets instantly alters intestinal microbiota towards a more pathogenic composition. The first line of defense against luminal bacteria is established by the mucus layer. If this physical barrier is impaired, bacteria can weaken intestinal integrity more effectively. Another defense mechanism in the intestine is constituted by the immune system. Especially CD4 T cells maintain a continuous cross-talk with the microbiota. T_H17 cells, crucial players at sites exposed to microorganisms, exhibit pathogenic and tissue regenerative effects. Our study focuses on mucus changes caused by short-term western-type diet (WTD). Additionally, we aim to elucidate functional alterations in CD4 T cells during relapsing/remitting dietary interventions.

Methods and results: $Foxp3^{RFP}IL\text{-}17A^{Kata}IL\text{-}10^{GFP}IL\text{-}22^{PacBlue}$ reporter mice allowed us to analyze T_{reg} and T_H17 cells in mucosa-associated lymphoid tissues (MALT). Our data indicated that 3-day WTD consumption results in reduction of IL-10 secretion from T_{reg} and T_H17 cells in Payer's patches by trend. While T_{reg} recovered IL-10 secretion after mice were switched back to chow diet, release of IL-10 by T_H17 cells decreased further. Conversely, in mesenteric lymph nodes, T_H17 cells display higher IL-10 secretion after the chow recovery phase. Additionally, after short-term WTD, mucus was more toxic to $CD45^+$ lymphocytes. It is still under investigation whether mucus toxicity depends on WTD-dependent microbial adaptations or whether it can be directly attributed to WTD.

Discussion/Conclusion: Considering all preliminary results, we hypothesize that in states of disrupted epithelial barrier integrity, the resulting proximity of toxic mucus to underlying immune cells might generate a transient immunodeficient barrier, thereby causing an uncontrolled bacterial invasion. Furthermore, as MALT-resident CD4 T cells might be affected in their regulatory capacity, we envision that such alterations predispose to chronic inflammatory bowel disease, especially upon repeated exposure to WTD.

Knockout of endocannabinoid receptor 1 reduces hepatic steatosis in a mouse model of chronic hepatitis B

F. Schneider, K. Irungbam, Y. Churin, M. Roderfeld, E. Roeb
Gastroenterology, Medical Clinic II, Justus-Liebig-University Giessen, Germany

Introduction: Transgenic mice expressing the hepatitis B surface antigen (HBsAg) develop a liver fibrosis but also a hepatocellular carcinoma at the age of one year. The cannabinoid receptor 1 (CB1) is involved in controlling processes of liver inflammation. In this project the influence and therapeutic impact of a CB1 receptor knockout on lipid metabolism in HBV transgenic mice was examined.

Methods: HBV transgenic mice on BALB/c genetic background were crossed with a CB1-knockout mice (HBVtg/CB1-ko) and killed at the age of 26 and 52 weeks. Wildtype BALB/c mice (Wt), HBV transgenic mice (HBVtg) and CB1-knockout mice (CB1-ko) of the same age were examined as controls. Body weight and serum parameters were investigated and liver samples were analyzed via immunohistochemistry, microarray, quantitative real time-PCR (qRT-PCR) and thin layer chromatography.

Results: HBVtg mice show an age-dependent increase of hepatic steatosis caused by augmented triglyceride accumulation. By the knockout of the CB1 receptor in HBVtg mice the triglyceride accumulations and also the serum glucose levels were reduced. The dysregulation of metabolic pathways and enzymes as well as the partial loss of hepatic zonation in HBVtg mice could be improved. Furthermore, an increased gene expression of Perilipin-2 (involved in lipid droplet formation) and MOGAT1 (involved in triglyceride synthesis) was observed und improved by the knockout of the CB1 receptor.

Discussion/Conclusion: The knockout of the cannabinoid receptor 1 in HBVtg mice reduced age-dependent hepatic steatosis. The synthesis of lipids as well as the capacity of lipid storage were decreased which probably leads to an improved hepatic steatosis.

32

A novel mouse model to study IL-6/gp130 signaling and acute-phase proteins in the gut-liver axis

Neele Schumacher[1], Miryam Müller[1], Karsten Lücke[2], Thomas Wunderlich[3], Hanna Lotter[4], Nicola Gagliani[5], Hans-Willi Mittrücker[2], Ateequr Rehman[6], Stefan Rose-John[1] and Dirk Schmidt-Arras[1]
[1]Institute of Biochemistry, Christian-Albrechts-University Kiel, Germany
[2]Institute of Immunology, University Medical Center Hamburg-Eppendorf, Germany
[3]Max-Planck Institute for Metabolism Research Cologne, Germany
[4]Department of Molecular Parasitology, Bernhard-Nocht Institute Hamburg, Germany
[5]Center for Internal Medicine, University Medical Center Hamburg-Eppendorf, Germany
[6]Institute of Clinical Molecular Biology, Christian-Albrechts-University Kiel, Germany

Introduction: Interleukin 6 (IL-6) is a pleiotropic cytokine involved in inflammation and tissue regeneration. In complex with its cognate IL-6 receptor it binds to the signal-transducing subunit gp130. In the liver and the gut, IL-6 has been shown to be crucial for the induction of acute-phase proteins (APP), liver regeneration, but also tumour formation. Recently, IL-6 was linked to microbial dysbiosis in a DSS colitis model.

Methods: We recently generated ROSA26-transgenic mice allowing for the Cre-inducible expression of a constitutive active gp130 variant (Lgp130). These mice were bred to Alb-CreERT2 or Vil-CreERT2 mice to allow tamoxifen-inducible expression of Lgp130 in hepatocytes or intestinal epithelial cells. Induction of APPs was analysed by qPCR and ELISA. Consequences of elevated APPs on inflammatory cells was assessed by flow cytometry and immunohistochemistry, whereas alterations in the gut microbiome were analysed by microbiome sequencing.

Results: We demonstrate that Lgp130 expression markedly induces expression of APPs either systemically (Alb-CreERT2) or locally (Vil-CreERT2). Systemic elevation of APPs by hepatocytic gp130 signals protects from *Listeria monocytogenes* infection, while intestinal gp130 signals altered the gut microbiome.

Discussion/Conclusion: Taken together, our model allows for the first time the analysis of gp130 signaling exclusively in hepatocytes or intestinal epithelial cells and its consequences on intestinal inflammation and the gut microbiome.

Xanthohumol, a prenylated chalcone derived from hops, inhibits hepatic metastasis of tumor cells

Tatjana Seitz[1], Peter Dietrich[1], Christina Hackl[2], Abdo Mahli[1], Sven Arke Lang[3], Anja Katrin Bosserhoff[1,4], Claus Hellerbrand[1,4]
[1]Institute of Biochemistry (Emil-Fischer-Zentrum), Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany
[2]Department of Surgery, University Hospital Regensburg, Germany
[3]Department of Surgery, University of Freiburg, Germany
[4]Comprehensive Cancer Center Erlangen, CCC Erlangen-EMN, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany

Introduction: In many cancer entities, including melanoma, hepatic metastasis is the critical factor determining the clinical course and the mortality of the patient. Xanthohumol, a prenylated chalcone derived from hops, is known to exert a broad range of antitumor activities. However, its functional effects on melanoma cells had not been investigated and no *in vivo* studies of Xanthohumol effects on metastasis existed. The aim of this study was to investigate XN effects on (hepatic) metastasis of melanoma cells.

Methods: XN effects on proliferation, migration and colony formation of human melanoma cell lines were analyzed *in vitro*. Moreover, we tested *in vitro* findings in a syngeneic *in vivo* model of hepatic metastasis (splenic injection of B16 melanoma cells in C57/BL6 mice). Subcutaneously implanted pellets (Innovative Research of America) released XN with a daily dose of 10 mg/kg body weight. Controls received placebo pellets. Liver weight, histological analysis and expression of MIA, a gene specifically expressed in melanoma cells, were used to measure hepatic tumor burden.

Results: Xanthohumol exhibited cytotoxic effects on melanoma cells beginning in the dose-range of 40–60 µM. Incubation with 10-fold higher Xanthohumol concentrations did not impair the viability of primary human hepatocytes. Interestingly, incubation with Xanthohumol in the subtoxic range inhibited proliferation, migration and colony formation of melanoma cells *in vitro*. In the murine model, liver weight and hepatic MIA expression were significantly decreased in Xanthohumol treated mice. Furthermore, hepatic metastases of Xanthohumol treated mice revealed significantly larger areas of central necrosis and lower Ki67 expression compared to control mice.

Discussion/Conclusion: Xanthohumol inhibits tumorigenicity of melanoma cells *in vitro* and significantly reduced hepatic metastasis of melanoma cells in mice. These data suggest Xanthohumol as a promising agent for the treatment of hepatic (melanoma) metastases.

34

Effect of diet, physical activity, sleep and defecation on quality of life

K.A. Shemerovskii
Institute of Experimental Medicine, St. Petersburg, Russia

Introduction: It is known that such diurnal factors as diet, physical activity, sleep and defecation affect the composition of microbiota and human health. However, the partial contribution of each of these factors remains unclear.
The purpose of this study was a comparative analysis of the partial contribution of each of diurnal factors to the quality of life.

Methods: Chronoenterography (monitoring of the circadian rhythm of the defecation), and quality of life (QoL) questionnaire, taking into account the level of satisfaction with diet, physical activity and sleep in 148 gastroenterologists were used. QoL in persons with a regular bowel movement (RBM) – 7 times a week (diurnal rhythm) was compare with irregular bowel movements (IBM) – 1–6 times a week. Satisfaction by level of diet, activity and sleep were evaluated according to the 5-point system: 5 points – 80–100% satisfaction (optimal), 1 point – satisfaction up to 20% from the optimal (low).

Results: It was shown that improvement of satisfaction with the diet from the "good" level (60–80% of optimal) to the "excellent" level (80–100% of optimal) improved the quality of life by 15%.
Improving of satisfaction by physical activity from "good" to "excellent" contributed to an improvement of QoL by 18%.
Improving of satisfaction by sleep from "good" to "excellent" increased the level of quality of life by 19%.
Improving of satisfaction with the circadian rhythm of defecation from IBM to RBM increased the level of QoL by 48%.

Discussion/Conclusion: The partial contribution of defecation frequency (48%) into quality of life practically corresponded to the sum of the partial contributions of such three diurnal factors as diet (15%), physical activity (18%) and sleep (19%).

Epithelial cell-derived complement component 3 is involved in intestinal immune responses during chronic colitis

Kerstin Skibbe[1,§], Annika Sünderhauf[1,§], Sophie Preisker[1], Karen Ebbert[1], Admar Verschoor[2], Christian M. Karsten[2], Claudia Kemper[2,3,4], Marijana Basic[5], André Bleich[5], Christian Sina[1], Stefanie Derer[1]
[1]Institute of Nutritional Medicine, Molecular Gastroenterology, University Hospital Schleswig-Holstein, Campus Lübeck, Germany; [2]Institute for Systemic Inflammation Research, University of Lübeck, Germany; [3]Division of Transplant Immunology and Mucosal Biology, Medical Research Council Centre for Transplantation, King's College London, Guy's Hospital, Great Maze Pond, London SE1 9RT, UK; [4]Laboratory of Molecular Immunology and the Immunology Center, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA; [5]Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School, Hannover, Germany
[§]These authors contributed equally to this work.

Introduction: The complement system is part of innate sensor and effector systems, which recognize and quickly respond to microbial-associated molecular patterns (MAMPs) with a tailored defense reaction. MAMP recognition and appropriate immune responses are of major importance for the maintenance of intestinal barrier function. Hence, we studied the crosstalk between intestinal epithelial cells (IEC), bacteria and the complement system in the course of chronic colitis.

Methods: Chronic colitis was induced in C57BL/6JRj mice through administration of 2% w/v dextran sodium sulphate (DSS) in 3 cycles of 5 days intermitted by 5 days of water. IEC mRNA expression was measured by real-time PCR (RT-PCR) and immunohistochemistry was performed to determine intestinal and luminal localization of complement components. Ileal and colonic mucosa-associated bacteria (MAB) isolated from WT, TLR4$^{-/-}$ or germ-free mice were analyzed by flow cytometry. Expression of colonic complement components was studied by TaqMan RT-PCR in IBD patients as well as healthy individuals.

Results: Our data demonstrate that IECs constitutively produce C3 and express C3aR and TLRs1–4. C3 expression patterns differed between ileum and colon, with significantly higher expression levels in the colon. This spatial difference was induced or maintained through commensal bacteria. In addition, TLR4-mediated signals in IECs induced increased C3 expression and C3a generation, which in turn led to an enhanced ratio of pro- to anti-inflammatory cytokines. We confirmed these findings in primary IEC from chronic DSS-induced colitis models. High amounts of C3 were detected in cryptic IECs and murine mucus samples, leading to opsonization of MAB with C3 fragments. Notably, in contrast to CD patient samples, we did not observe upregulation of C3 mRNA expression in inflamed colonic biopsy samples from UC patients – pointing to an impaired intestinal complement system in UC.

Discussion/Conclusion: These findings demonstrate a pivotal role of the TLR4-C3 axis in intestinal immune response during chronic colitis.

36

Analysis of the effects of ethanol on expression of factors promoting hepatic metastasis in hepatoma and melanoma cells

Judith Sommer, Kim Freese, Claus Hellerbrand
Institute of Biochemistry, Emil-Fischer-Zentrum, Friedrich-Alexander University Erlangen-Nuremberg, Germany

Introduction: The liver is an attractive "soil" for metastasis of several primary tumors, including malignant melanoma. However, the molecular mechanisms and risk factors that affect hepatic metastasis are mostly unknown. (Chronic) alcohol consumption induces pathological changes in the liver, including enhanced expression of growth factors and pro-angiogenic factors known to induce tumor progression. Hereby, the main cause is oxidative stress, which is at least partly induced through ethanol metabolism by CYP2E1.

The aim of this study was to analyze the effect of ethanol on factors promoting hepatic metastasis *in vitro*.

Methods: Conditioned medium (CM) was generated from the human hepatoma cell lines HepG2-E47 (stably expressing CYP2E1) and HepG2-C34 (not expressing CYP2E1) stimulated with ethanol (50 or 100 mM) for 24 h or control medium. Subsequently, human melanoma cell lines were exposed to CM of control and ethanol-treated HepG2-E47 and HepG2-C34 cells, as well as ethanol (50 or 100 mM) alone. Cell proliferation was detected by electrical impedance measurement applying the xCELLigence system (Roche). Additionally, mRNA levels of genes relevant for melanoma metastasis were analyzed in ethanol-treated (125 or 250 mM) melanoma cells by qPCR.

Results: CM of ethanol-treated HepG2-E47 cells induced a higher proliferation rate in melanoma cells than control CM or ethanol stimulation alone. Interestingly, also CM from ethanol-treated HepG2-C34 cells had reduced effect on melanoma cell proliferation compared to CM from ethanol-treated HepG2-E47 cells. Still, also ethanol stimulation alone induced proliferation of melanoma cells compared to cells in control medium. Furthermore, exposure to ethanol significantly induced expression levels of VEGF, ICAM-1 and RANTES in HepG2-E47 hepatoma cells and also this effect was reduced in HepG2-C34 cells.

Discussion/Conclusion: Our in vitro data suggest that ethanol induces different mechanisms that affect (hepatic) metastasis. In hepatocytes, it induces the secretion of pro-mitogenic factors. Hereby hepatic ethanol metabolism by CYP2E1 is likely to play a central role. Furthermore, ethanol directly induces proliferation of melanoma cells. In vivo models and epidemiological studies are needed to further investigate the connection between alcohol consumption and (hepatic) metastasis, which would have important implication for the guidance and recommendations for tumor patients.

Analysis of the interaction between inflammatory bowel disease and liver pathology in a murine model

Niklas Steffens[1], Tanja Bedke[1], Samuel Huber[1]
I. Medizinische Klinik und Poliklinik Universitätsklinikum Hamburg-Eppendorf, Germany

Introduction: Primary sclerosing cholangitis (PSC) is a cholestatic liver disease leading to strictures of the extra- and intrahepatic bile ducts. PSC is strongly associated with inflammatory bowel disease (IBD) especially with ulcerative colitis (UC). The prevalence of IBD in PSC ranging from 67–73%. The reason for this association remains elusive. The DDC (3,5-diethoxycarbonyl-1,4-dihydrocollidine) model and the *Mdr2* (Abcb4)$^{-/-}$ mice are established mouse models to investigate cholestatic liver disease like PSC. These mice develop severe liver fibrosis with a PSC like phenotype. Using different colitis models we tried to investigate the interaction between IBD and PSC.

Methods: To analyse the relationship between IBD and PSC we chemically induced acute and chronic colitis using dextran sodium sulfate (DSS) in *Mdr2*$^{-/-}$ mice and the DDC mouse model. As an alternative model of intestinal inflammation we used *Citrobacter rodentium* infection in *Mdr2*$^{-/-}$ mice. We performed FACS analysis, histopathology scoring and analysis of liver transaminases to assess the severity of liver damage and characterise the different cell populations.

Results: After the induction of acute and chronic DSS colitis in *Mdr2*$^{-/-}$-mice and acute DSS colitis in DDC fed mice we observed an infiltration of T_H1/T_H17, T_H17 and innate immune cells into the liver. Interestingly DSS treated mice showed reduced liver transaminases. After the infection of *Mdr2*$^{-/-}$-mice with *Citrobacter rodentium* we only saw an infiltration of T_H17-Zells into the liver. The pre-existing PSC like phenotype was not affected by the infection.

Discussion/Conclusion: Intestinal inflammation seems to promote the infiltration of proinflammatory cell populations into the liver. Interestingly DSS treatment of *Mdr2*$^{-/-}$ mice and DDC fed mice reduced liver transaminases. This was not the case in a bacterial driven model of intestinal inflammation.

38

Saccharin supplementation modulates the intestinal microbiome and is protective in early stages of experimental colitis

A. Sünderhauf[1], A. Künstner[2,3], R. Pagel[1,4], A.E. Wagner[1], S. Ibrahim[5], S. Derer[1], C. Sina[1]
[1]Institute of Nutritional Medicine and Medical Department 1, University Hospital Schleswig-Holstein, Campus Lübeck, University of Lübeck, Germany
[2]Group for Medical Systems Biology, Lübeck Institute of Experimental Dermatology, University of Lübeck, Germany
[3]Institute of Cardiogenetics, University of Lübeck, Germany
[4]Institute of Anatomy, University of Lübeck, Germany
[5]Lübeck Institute of Experimental Dermatology, University of Lübeck, Germany

Introduction: Inflammatory bowel disease (IBD) comprises a group of chronic inflammatory disorders of the human gastrointestinal tract with rising incidence in western societies. A microbial dysbiosis of the gut due to a “western diet” and a high intake of artificial sweeteners are discussed as potential triggers for IBD. However, the effect of an artificial sweetener such as saccharin has yet not been tested in a murine experimental colitis model.

Methods: Initially, effects of saccharin on bacterial growth were analysed *in vitro*. *In vivo*, mice received either drinking water supplemented with saccharin or drinking water alone for five weeks. Subsequently, experimental chronic colitis was induced by a cyclic administration of 2% (w/v) dextran sodium sulphate (DSS) via the drinking water. Extend of inflammation was assessed by the disease activity index (DAI) and immunohistochemistry analyses. Faecal samples were collected and subjected to microbiome analysis via next generation sequencing and mucosa-attached bacteria (MAB) were quantified and characterized by flow cytometry analyses.

Results: *In vitro* bacterial growth was inhibited in the presence of high doses of saccharin. Non-DSS saccharin-supplemented mice depicted significant alterations of the gut microbiome as well as MAB composition. Furthermore, both faecal bacterial and MAB load were significantly decreased in these mice, while defensin expression in the ileum was upregulated. Surprisingly, saccharin prefeeding of mice resulted in protective effects on intestinal inflammation during the first 20 days of the DSS-induced colitis.

Conclusion: Contrasting our primary hypothesis, these data suggest saccharin supplementation as being preventive in the acute phase of colitis by lowering the faecal as well as mucosa-attached bacteria load.

Linking mucus depletion and microbiome alterations in UC to mucosal energy supply

A. Sünderhauf[1], F. Bär[1], M. Hirose[2], A. Künstner[3,4], R. Pagel[1,5], S. Ibrahim[2], S. Derer[1], C. Sina[1]
[1]Institute of Nutritional Medicine and Medical Department 1, University Hospital Schleswig-Holstein, Campus Lübeck, University of Lübeck, Germany
[2]Lübeck Institute of Experimental Dermatology, University of Lübeck, Germany
[3]Group for Medical Systems Biology, Lübeck Institute of Experimental Dermatology, University of Lübeck, Germany
[4]Institute of Cardiogenetics, University of Lübeck, Germany
[5]Institute of Anatomy, University of Lübeck, Germany

Introduction: Mucosal ATP depletion as the consequence of reduced mitochondrial respiratory chain activity has been attributed to ulcerative colitis (UC) pathogenesis more than 30 years ago. Mucus producing goblet cells, which are highly dependent on energy supply via the oxidative phosphorylation (OXPHOS) system, are supposedly highly affected by reduced OXPHOS activity. Furthermore, intestinal mucus depletion has been suggested as a histological hallmark of UC. Finally, mucus composition and glycosylation is able to shape the intestinal microbiota, which is frequently altered in UC patients. Nevertheless, experimental evidence linking mitochondrial dysfunction with intestinal mucus depletion and microbiome alterations are still missing.

Methods: Mucus producing HT29MTX cells as well as murine intestinal organoids are employed as *in vitro* or *ex vivo* model systems, respectively, in order to study mucin transcription, production, secretion and glycosylation upon energy metabolism modifying stimuli. *In vivo*, conplastic mice, carrying well characterized single nucleotide polymorphisms in their mitochondrial genome leading to altered OXPHOS function, are studied on their susceptibility to various experimental colitis models. Furthermore, seahorse analyzer is used to control for mitochondrial respiration and glycolysis, while microbiome composition is analysed via 16srRNA next generation sequencing.

Results: *In vitro* stimulation of HT29MTX cells showed that mucus transcription and secretion is highly dependent on mitochondrial OXPHOS capacity. In line with this, mice with reduced mucosal ATP levels and OXPHOS activity depicted goblet cell reduction and increased susceptibility to DSS colitis, characterized by elevated DAI, MEICS and histologic scores. On the other hand, mice with increased mucosal ATP levels were protected against experimental colitis. Finally, gut microbial composition was highly dependent on mitochondrial genetics.

Conclusion: Taken together, we here describe a new pathway linking major hallmarks of UC. Mitochondrial OXPHOS activity and mucosal ATP levels in intestinal epithelial cells might be strongly underestimated factors in UC pathogenesis and therapeutic potentialities.

40

Possible common genetic background for metabolic and immune disorders in hepatic steatosis, obesity, and hypertension

A.R. Sydorchuk, L.P. Sydorchuk, O.M. Plehutsa, Y. Yarynych, R.I. Sydorchuk, I.I. Sydorchuk
Bukovinian State Medical University, Chernivtsi, Ukraine

Introduction: Hepatic steatosis (HS), obesity and arterial hypertension (AH) have multiple common mechanisms of development involving metabolic and immune changes. Among them, impairment or inhibition of receptor molecules controlling enzymes responsible for oxidation and fatty acids synthesis, appear to contribute to fat accumulation. However, HS is to some extend still accepted as a non-systemic pathology, rather than systemic disease, not limited to liver but involving interaction between genetically determined mechanisms of metabolism regulation and systemic inflammation. We hypothesized that there is possible genetically based background for both immune and metabolic changes under these conditions. The aim of this study was to investigate the role of several candidate gene's; single nucleotide polymorphisms (Pro12Ala polymorphism of peroxisome proliferator-activated receptor-gamma [PPAR-γ2] gene and insertion/deletion [I/D] polymorphism of angiotensin converting enzyme [ACE] gene) on metabolic profile and cytokines in obese patients with common combination of HS and AH.

Methods: Study included 154 HS patients with AH (87 male, 67 female, age 50.06 ± 7.34). Duration of HS 1–5 years, AH 3–21 years. NAFLD/NASH diagnosis and management according to EASL and AGA/AASLD/ACG guidelines. Metabolic disorders were defined with body mass index (BMI), glycemia, immunoreactive insulin (IRI), total cholesterol (TC), low- and high-density cholesterol (LDL-C, HDL-C), triglycerides (TG), C-peptide (CP) levels and HOMA-IR index. TNF-α and leptin plasma levels were assessed by ELISA. Genes' polymorphism of PPAR-γ2 (Pro12Ala), and ACE (I/D) alone or in combination studied with PCR.

Results: Differences of BMI, plasma glucose, IRI, HOMA-IR, CP and leptin are independent from ACE gene genotypes ($p > 0.05$). Pro-allele carriers of PPAR-γ2 gene have higher BMI than AlaAla carriers (32.7 ± 2.1 and 27.9 ± 1.1 kg/m^2 vs. 25.6 ± 0.8 kg/m^2, accordingly [$p < 0.05$]); leptin level – 14.3 ± 0.41 and 8.6 ± 0.25 ng/ml vs. 3.7 ± 0.22 ng/ml ($p < 0.001$); glucose level – by 10.2% and 10.9%, accordingly ($p < 0.05$); CP level was higher in ProPro-genotype than in Ala-allele carriers by 15.7% ($p < 0.05$). Risk group for dyslipidemia are ProPro-genotype carriers of PPAR-γ2 gene with higher level of TC, TG and LDL-C by 16.4%, 17.3% and 27.9% ($p < 0.05$) and lower level of HDL-C in women by 25.6% ($p = 0.038$). Lipids levels are independent on ACE I/D polymorphism. Baseline TNF-α plasma levels did not significantly deviate between genotypes of PPAR-γ2 gene, but D-allele carriers (I/D+DD) of ACE gene had higher baseline TNF-α plasma levels (91.61 pg/ml and 109.11 pg/ml, accordingly [$p < 0.01$]).

Discussion/Conclusion: Metabolic disorders in HS obese hypertensive patients are associated with PPAR-γ2 Pro-allele (carbohydrates) and ProPro-genotype (lipids). Presence of D-allele of ACE gene is associated with reliably higher TNF-α plasma levels. These pathologic conditions have similar genetically determined pathogenetic mechanisms involving combination of impaired metabolism and immunity.

41

ACE (I/D) and AGTR1 (A1166C) genes single nucleotide polymorphisms, non-alcoholic fatty liver disease and intestinal dysbiosis

L.P. Sydorchuk, R.I. Sydorchuk, O.V. Kushnir, O.M. Iftoda, A.R. Sydorchuk,
I.I. Sydorchuk, O.M. Plehutsa, O.Y. Khomko
Bukovinian State Medical University, Chernivtsi, Ukraine

Introduction: Non-alcoholic fatty liver disease (NAFLD) is the most common liver disorder in developed countries (6–35%) with about 30% prevalence in the United States between 2011–2012 as shown in the recent study using the National Health and Nutrition Examination Survey. Obesity, diabetes (DM), hypertension (AH), and other systemic diseases frequently combine with NAFLD due to multiple similar pathogenesis mechanisms. The aim of this study is to find possible connections between intestinal dysbiosis, hemodynamic peculiarities of mesenteric vessels, NAFLD and ACE (I/D) and AGTR1 (A1166c) genes' polymorphisms.

Methods: Study involves 104 patients: 50 (48.1%) women, and 54 (51.9%) men. Mean age – 53.2 ± 8.7. NAFLD, AH, and DM diagnosis and management according to AASLD/ACG/AGA, ASC/ESH and ASD Guidelines, respectively. Microbiology included taxonomic group identification and population levels determination with 33.3% variation as dysbiosis single step deviation. Visceral blood vessels status evaluated sonografically. Genetic polymorphisms studied by PCR.

Results: Ischemic changes in mesenteric blood vessels were characterized by decrease of time overage velocity (reliable in D-allele carriers of ACE gene and C-allele of AGTR1 gene in 1.4–1.94 times, $p < 0.05$), increase of peak systolic and end diastolic velocity (not depending on genotypes of analysed genes in 1.5–3.05 times, $p < 0.05$) and peripheral resistance by Gosling index (2–2.35 times, $p < 0.05$). Dysbiosis severity strongly correlated with NAFLD, AH severity in D (ACE) and A (AGTR1) allele's carriers while changes in blood vessels correlated with dysbiosis severity with weaker dependence on genotypes. AGTR1 gene's CC-genotype carriers had the highest risk of abdominal vessels changes. Severity of dysbiosis moderately and strongly $r = 0.61–0.83$, $p \leq 0.04–0.001$) positively correlated with NAFLD, AH and DM severity.

Discussion/Conclusion: While genetic predisposition to NAFLD is considered to be proved, the role of genetic factor explaining systemic changes in NAFLD patients is controversial. Both ACE and AGTR1 genes' polymorphisms has no direct influence on liver but determine changes of blood vessels and intestinal microbiota. It causes respective influence on metabolic profile with reliable correlation of dysbiosis and disease severity.

Intestinal barrier suffer major impact in acute enteral dysfunction syndrome due to gut microflora, antiendotoxin core antibodies and nitric oxide associated vicious circle

R. Sydorchuk, L. Sydorchuk, O. Kolomojets, O. Plehutsa, I. Sydorchuk, O. Khomko, A. Sydorchuk
Bukovinian State Medical University, Chernivtsi, Ukraine

Introduction: Enteral dysfunction syndrome (EDS) is the leading course of hospital-associated mortality in Europe and is characterized by increased intestinal permeability, hepatic dysfunction and multiple organ dysfunction syndrome (MODS), and different distant complications. It has multiple clinical presentations as well as different causes and outcomes. Pathogenesis of EDS is complex and to some extent, unclear. We hypothesized that microbiota may participate in this malicious status by means of a vicious circle which includes changes of gut microbiocenosis with associated Antiendotoxin Core Antibodies (EndoCAb) and nitric oxide (NO) changes. The objective of the study is to find whether changes of gut microflora, EndoCAb and nitric oxide levels are somehow related in pathogenesis of EDS.

Methods: Study included 87 patients with clinically proven EDS, mean age – 49.06 ± 8.34 years. Control – 30 practically healthy individuals of respective age and gender. EndoCAb assessed by ELISA, NO (nitrite/nitrate) by IEA. Colonic lumen and mucosal microflora studied microbiologically.

Results: Colonic flora changes dramatically in EDS: significant decrease ($p < 0.05$) or elimination of autochthonic anaerobic microorganisms and hyperproliferation of conditionally pathogenic Enterobacteriacea: E.coli, including Hly+ – 9.31 ± 0.62 lg CFU/g against 7.39 ± 0.56 lg CFU/g in control; Klebsiellae – 5.17 ± 0.40 lg CFU/g against 3.48 ± 0.49 lg CFU/g in control; Proteus – 6.23 ± 0.35 lg CFU/g, and Serratia – 5.49 ± 0.74 lg CFU/g (not found in control). EndoCAb changes were not uniform. In patients with negative (complicated or acute lethal) clinical course of disease EndoCAb IgM (1.05 ± 0.02 MMU/ml) and IgG (2.51 ± 0.11 GMU/ml) were significantly lower than in control ($p < 0.01$). However, positive current of the syndrome, even accompanied by MODS, gives higher figures of IgM (2.98 ± 0.23 MMU/ml) and IgG (9.57 ± 0.84 GMU/ml). In most cases (83–95.4%) significant ($p < 0.05$) EndoCAb growth was observed only week of disease. In four (4.6%) cases only IgM increased, while IgG level remained low. NO levels rose reliably ($p < 0.05$) in all observed patients with EDS (42.96 ± 2.75 mmol/l vs. 34.61 ± 3.07 mmol/l in healthy subjects). Strong negative correlation ($r = -0.79$, $p < 0.05$) between EndoCAb and NO levels was found only in negative course cases.

Discussion/Conclusion: Excessive growth of conditionally pathogenic Enterobacteriacea and endotoxin release is associated with insufficient production of antinuclear antiendotoxin antibodies (EndoCAb). NO aggravate EDS severity by means of decreased motility and increased gut' permeability. This "intestinal leakage" plays role as pathophysiologic vicious circle forming conditions for microbiome changes and refractory changes of associated chains of pathogenesis.

43

IgA antibodies against filamentous-actin are frequently detected in patients with cirrhosis and indicate a progressive disease course

Tamas Tornai[1], Boglarka Balogh[1], Nora Sipeki[1], Zsuzsanna Vitalis[1], Istvan Tornai[1], Peter Antal-Szalmas[2], Tamas Dinya[3], Gary L. Norman[4], Tony Bruns[5,6], Maria Papp[1]
[1]Division of Gastroenterology, Department of Internal Medicine, [2]Department of Laboratory Medicine, [3]Institute of Surgery, Faculty of Medicine, University of Debrecen, Hungary; [4]Inova Diagnostics, Inc., San Diego, California, USA; [5]Department of Internal Medicine IV, University Hospital Jena, Germany; [6]Center for Sepsis Control and Care, University Hospital Jena, Germany

Introduction: Gut barrier failure and the consequential pathological bacterial translocation (BT) are characteristic features of cirrhosis and play an important role in the progression of liver disease. We hypothesized that serological hallmarks of gut barrier dysfunction are associated with accelerated progression of liver disease in cirrhosis and the development of specific complications and liver-related death.

Methods: Sera from 260 stable outpatients with cirrhosis (male: 129, age: 56 ± 11 years, alcohol: 167 [64.2%]) and from 155 healthy subjects were assayed for the presence of antibodies against filamentous-actin (AAA IgA and IgG) and gliadin (AGA IgA and IgG) and for intestinal fatty acid-binding protein (I-FABP) by ELISA. Association of gut failure markers with disease specific characteristics was assessed at baseline and the course of liver disease was evaluated in a 5-year follow-up observational study for decompensating events (ascites, variceal bleeding, hepatic encephalopathy and/or bacterial infection) and liver-related death. BT was assessed based on the presence of anti-microbial antibodies (anti-OMP plus IgA and/or endotoxin core IgA antibody [EndoCab]).

Results: Elevated concentrations of the gut failure markers IgA-AAA (62.7 vs. 4.4%) and IgA-AGA (27.7 vs. 2.6%) were more often observed in cirrhosis as compared to healthy controls ($p < 0.001$ for both). In addition, serum I-FABP was increased in cirrhosis as compared to controls (741 vs. 244 pg/ml, $p < 0.001$) and correlated with serum levels of IgA-AAA and IgA-AGA. IgA-AAA positivity was associated with alcoholic liver disease, liver disease scores and decompensated clinical stage (all $p < 0.001$). Serological markers of BT were more often found in patients with elevated IgA-AAA compared to those without (72.3 vs. 13.5 % for IgA-EndoCab and 85.2 vs. 20.5% for IgA-anti-OMP, $p < 0.001$ for both). In patients with compensated disease stage (n = 131) the risk of decompensation was higher in patients with elevated IgA-AAA (HR = 1.85; 95% CI: 1.06–3.24), as was the risk of liver-related mortality (HR = 2.66; 95% CI: 1.27–5.56). Such associations were not observed for IgG-AAA and IgA/IgG-AGAs. In the overall cohort, IgA-AAA remained an independent predictor of liver-related death (HR_{adj} = 1.96; 95% CI: 1.08–3.55]) when adjusting for important clinical variables (MELD score, etiology, clinical stage, see **Table 1**).

Table 1. Summary of Cox proportional hazards analysis in the prediction of liver-related mortality.

n = 260	Wald	Hazard ratio	95% confidence interval	p-value
AAA IgA positivity (≥ 35 U)	4.83	1.96	(1.08–3.58)	0.028
MELD score (per 1 point increase)	11.71	1.10	(1.04–1.17)	0.001
Etiology (alcohol)	1.63	0.70	(0.40–1.21)	0.202
Clinical stage (decompensated)	1.73	1.38	(0.86–2.22)	0.188

Discussion/Conclusion: Elevated serum concentrations of IgA antibodies against filamentous-actin indicate patients with an unfavourable outcome in cirrhosis, which may be related to intestinal damage beyond being related to bacterial translocation. IgA-AAA might be consider as a novel serologic marker of the disease progression.

44

Functional polymorphisms of innate immunity pattern recognition receptors do not constitute the risk of bacterial infections other than spontaneous bacterial peritonitis and also not the progressive disease course in patients with cirrhosis

Tamás Tornai[1], Tamás Dinya[2], Boglárka Balogh[1], Zsuzsanna Vitális[1], István Tornai[1], Dávid Tornai[3], Péter Antal-Szalmás[3], Hajnalka Andirkovics[4], András Bors[4], Attila Tordai[5] , Maria Papp[1]
[1]Division of Gastroenterology, Department of Internal Medicine, [2]Institute of Surgery, Faculty of Medicine, University of Debrecen, Hungary; [3]Department of Laboratory Medicine, [4]Laboratory of Molecular Diagnostics, Hungarian National Blood Transfusion Service, [5]Department of Pathophysiology, Semmelweis University, Budapest, Hungary

Introduction: Innate immunity pattern recognition receptors (PRRs) recognize distinct pathogen-associated molecular patterns (PAMPs) on the cell surface or in the cytoplasm. Functional polymorphisms of various PRRs have been established to contribute to susceptibility to spontaneous bacterial peritonitis (SBP). However, their role in the development of cirrhosis-associated bacterial infections (BI) beyond SBP remains unknown. We aimed to investigate the link between PRR gene variants, pathological bacterial translocation (BT) and thus the development of cirrhosis-associated complications.

Methods: 49 patients with cirrhosis (stable outpatients: 243 and acute decompensation: 106) were genotyped for the common *NOD2* (p.R702W, p.G908R and c.3020 insC), *TLR2* (g.6686T/A), *TLR4* (D299G) and *CD14* (c. 159C/T) gene variants. In the stable outpatients (male: 116, age: 56 ± 11 years, alcohol: 62.6%, ascites: 36.2%, MELD score: 11) incidence of BI, decompensating events (ascites, variceal bleeding and hepatic encephalopathy) and liver-related death were prospectively assessed in a 5-year follow-up observational study. BT was assessed based on the presence of anti-microbial antibodies (anti-OMP plus IgA and/or endotoxin core IgA antibody [EndoCab]) or increased serum level of lipopolysaccharide-binding protein (LBP).

Results: Ninety-four (38.7%) patients encountered at least one episode of BI. Distribution of bacterial infections were as follows: urinary tract infection (39.4%), SBP (27.7%), pneumonia (13.8%) and miscellaneous (24.5%). 5.3% of the cases were multifocal. PRR genetic profile was not associated with prior BI episode and also not with the risk of overall BI or infection-related death during the follow-up. *NOD2* variants, however, were associated with an increased cumulative probability of SBP in patients with ascites (n = 88) as compared to wild type (76.9% vs. 30.9%, p*LogRank* = 0.047). Frequency of anti-microbial antibodies and LBP levels did not differ between various PRR genotypes. Correspondingly, PRR genetic profile was not able to predict the long-term disease course in cirrhosis.

Discussion/Conclusion: In a Hungarian patient cohort with cirrhosis, common *NOD2* gene variants but not the other PRR polymorphisms enabled to improve identification of patients with high risk for SBP. None of the examined PRR gene variants influenced the risk of other types of BI or the long-term disease course. They were also not associated with serological markers of BT.

45

Single microRNA modulation in hepatocytes: A promising treatment for liver fibrosis

Hsin-Chieh Tsay[1,2,3], Qinggong Yuan[2,3], Asha Balakrishnan[2,3], Marina Kaiser[4], Marwa Farid[1,2,3,5], Selina Möbus[1,2], Andreas Kispert[4], Michael P. Manns[2], Michael Ott[2,3], Amar Deep Sharma[1,2]
[1]Junior Research Group MicroRNA in Liver Regeneration, Cluster of Excellence REBIRTH, Hannover Medical School, Hannover, Germany
[2]Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany
[3]TWINCORE, Centre for Experimental and Clinical Infection Research, Hannover, Germany
[4]Institute for Molecular Biology, Hannover Medical School, Hannover, Germany
[5]Human Cytogenetics Department, Human Genetics and Genome Research Division, National Research Center, Cairo, Egypt

Introduction: MicroRNAs have appeared as essential regulators of hepatic stellate cells activation during liver fibrosis. The functions of microRNAs in hepatic stellate cells have been intensively investigated; however, the importance of microRNAs in hepatocytes, the main cell population in the liver, during fibrosis is not yet fully addressed.

Methods: After screening hundreds of microRNAs in primary mouse hepatocytes to identify microRNAs as potential therapeutic regulators of liver fibrosis, we than applied inhibitors of the selected miRNA by adeno-associated virus *in vivo* during toxin-induced liver fibrosis. The effect of microRNA inhibition was examined by immunohisto-chemistry and immunofluorescence staining. The downstream target of microRNA was identified by *in silico* analyses, Western blot analysis, and dual-luciferase reporter assay.

Results: Inhibition of microRNA-221-3p specifically in hepatocytes limits the activation of hepatic stellate cells and reduces toxin-induced liver fibrosis by reducing secretion of C-C motif chemokine ligand 2 (CCL2), due to post-transcriptional regulation of G protein alpha inhibiting activity polypeptide 2 (GNAI2) by microRNA-221-3p.

Discussion/Conclusion: Collectively, our findings suggest that *in vivo* inhibition of microRNA-221-3p in hepatocytes ameliorates liver fibrosis, demonstrating that microRNAs modulation in hepatocytes, which are abundant and easy-to-target, may serve as one of the potential therapeutic approaches for treating liver fibrosis.

Nrf2 and c-Met on hepatocytes is protective during the development of non-alcoholic steatohepatitis (NASH)

L. Van den Burg, H.K. Drescher, S. Erschfeld, C. Trautwein, D.C. Kroy
Department of Internal Medecine III, University Hospital RWTH Aachen, Aachen, Germany

Introduction: NASH is one of the fastest growing medical problems and the third most common reason for liver transplantation in developing countries.
It is well known, that the anti-oxidative stress response is upregulated in NASH. Further, the HGF (hepatocyte growth factor) receptor c-Met (mesenchymal-epithelial transition factor) is known to play an important role in acute liver regeneration. However, the cell-specific role of c-Met on hepatocytes together with the constitutive deletion of the anti-oxidative stress marker Nrf2 during NASH development and fibrosis progression remains unclear.

Aim: This study investigates the role of c-Met in a conditional hepatocyte specific knockout model with the Albumin (Alb)-Cre system together with a constitutive knockout for Nrf2. Nrf2 is the key regulating transcription factor of the anti-oxidative stress response. c-Met$^{f/f}$Nrf2 and c-Met$^{\Delta Hepa}$Nrf2 mice were analysed in a mouse model of diet-induced steatohepatitis.

Methods: c-Met$^{f/f}$Nrf2 and c-Met$^{\Delta Hepa}$Nrf2 animals were fed HFD (high fat diet) for 24 weeks to induce steatohepatitis.

Results: After 24 weeks of HFD treatment c-Met$^{\Delta Hepa}$Nrf2 mice displayed an earlier and faster progressing steatosis by increased body weight, higher serum transaminases and severe histomorphological changes whereas c-Met$^{f/f}$Nrf2 animals retain an intact liver architecture. Those changes further resulted in an impaired glucose tolerance. After 24 weeks HFD feeding, c-Met$^{\Delta Hepa}$Nrf2 mice show an enhanced hepatic immune cell infiltration of neutrophils whereas interestingly the numbers of T cells are reduced.

Discussion/Conclusion: Hepatocyte specific c-Met deficiency together with a constitutive knockout for Nrf2 leads to a more severe phenotype in the HF-diet induced steatohepatitis model. This leads to the conclusion that there could be a direct link between the HGF/c-Met signalling pathway with the anti-oxidative stress response during NASH development.

47

German HCV(1b)-Anti-D cohort – Long-term history and treatment results

Manfred Wiese; East German HCV Study Group
Leberambulanz, Sektion Hepatologie, Universitätsklinikum Leipzig, Germany

Introduction: The long-term natural history of hepatitis C (1b) infection is still not fully understood. So far, there are few long-term studies with a known date of infection which include all those infected persons without bias. According to the initial reports it was assumed that after 20 years, 30% of patients would have developed liver cirrhosis but in the German Anti-D cohort the cirrhosis rate was much lower. A new issue is to find out to what extent the prognosis after nearly four decades has been influenced by the anti-HCV therapy.

Methods: Between August 1978 and March 1979 it came to the administration of 14 hepatitis C-contaminated anti-D immunoglobulin batches at 2867 East German women to prevent Rh isoimmunization. This HCV cohort is of particular interest because there are only a few cohorts with known HCV infection time that would allow precise statements about the spontaneous course and therapy results. Our data were collected in 15 study centers in East Germany since the beginning. The Leipzig anti D-cohort as part of the total cohort includes 356 women, of whom now 181 were followed up after 39 years. 79 women were treated with IFN-based therapies, 12 women with IFN-free regimen.

Results: After 39 years, 85% of the 181 women in the HCV ELISA were positive. 33% were viremic (HCV PCR-positive). Only 11 (8.3%) of viremic women had liver cirrhosis, 9 (5.4%) had advanced fibrosis. In the last 15 years a continuous, but slow rise of advanced fibrosis score was observed. HCC has not been diagnosed. 48 women with an IFN-based therapy and all women with an IFN-free regimen were treated successfully (SVR). Since 1978 six HCV RNA-positive women of the Leipzig cohort died; eight women died after viral clearance. The overall mortality in the therapeutic SVR group was lower than in the treatment-naïve group.

Discussion/Conclusion: Young women without comorbidity eliminate HCV (1b) infection spontaneously in approximately half of the infected cases. After 39 years, a continuous but relatively low progression with regard to final states such as cirrhosis, HCC or death could be confirmed in this cohort. IFN-free therapies should be recommended because all 12 cases responded and the overall mortality after successful therapy (SVR) was lower too.

Characterization of primary sclerosing cholangitis (PSC)-associated inflammatory bowel disease

Agnes Franziska Wittek, Babett Steglich, Samuel Huber
1. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Germany

Introduction: Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease of unknown origin. Almost 80% of patients suffering from PSC have concomitant clinically apparent inflammatory bowel disease (IBD). We hypothesized that PSC and IBD affect each other. First, we speculate that PSC might drive colon inflammation. Thus, all PSC-patients might have a subclinical colitis. Second, we hypothesized that PSC-associated IBD is different from Crohn's disease (CD) and ulcerative colitis (UC) on a molecular level.

Methods: To test these hypotheses, we collected colon biopsies from patients suffering from PSC, PSC-associated IBD, UC, CD, primary biliary cholangitis (PBC) and healthy controls. We quantified the RNA expression of pro- and anti-inflammatory cytokines. A paired colonic biopsy was used to assess colitis severity on a histological level.

Results: Interestingly most patients with PSC showed increased expression of pro-inflammatory cytokines such as *IL-22, IL-17* and *INFγ* in the intestine regardless whether they were diagnosed or not with IBD. However, *TGFβ* and *IL-10* were also increased in the colon in PSC patients without clinical manifest IBD compared to those with concomitant IBD. Furthermore, patients with PSC-associated IBD showed significant differences in several cytokines, including *TGFβ*, *IL-23*, and *IL-10,* compared to non-PSC associated IBD (UC and CD).

Discussion/Conclusion: In conclusion, most PSC patients seem to have an increased expression of pro-inflammatory cytokines compared to healthy controls even in the absence of clinical manifest IBD. Furthermore, we found some characteristic differences in the cytokine expression profile of PSC-associated IBD compared to UC and CD.

Author Index to Poster Abstracts

(Name - Poster Number)

Abatsieva, M.P. 2
Agalioti, T. 11, 30
Andirkovics, H. 44
Antal-Szalmas, P. 43, 44
Antonenko, A.V. 1

Bakulin, I. 2
Balakrishnan, A. 45
Balogh, B. 43, 44
Bang, C. 21
Bär, F. 27, 39
Barbora, Y. 6
Basic, M. 35
Bedke, T. 3, 11, 37
Belousova, L.N. 2
Beregova, T.V. 1
Betge, J. 9
Bleich, A. 35
Bohmann, S. 3
Bors, A. 44
Bosserhoff, A.K. 8, 10, 33
Böttcher, M. 25
Breitkopf-Heinlein, K. 4, 9
Brockmann, L. 11
Bruns, T. 43

Cai, C. 4, 9
Castro, J.P. 13
Chiet, A. 22
Churin, Y. 12, 14, 15, 24, 31
Coleman, D. 13

Davies, N. 23
Derer, S. 27, 35, 38, 39
Dietrich, P. 8, 10, 33
Dinya, T. 43, 44
Dooley, S. 23
Dorbath, D. 16
Doulberis, M. 19
Drescher, H. 5, 46
Dvorak, K. 6

Ebbert, K. 35
Ebert, M.P. 4, 9
Eckert, T. 14
Erschfeld, S. 46

Farid, M. 45
Fillipidis, G. 17
Fischer, A. 30
Franke, A. 21
Freese, K. 7, 36
Fritz, V. 8
Fromann, L. 30

Gagliani, N. 3, 11, 30, 32
Gaitantzi, H. 4, 9
Garcia Perez, L. 11
Gaza, A. 10
Geier, A. 16
Gellert, F. 13
Giannou, A. 11
Glebe, D. 14
Grevelding, C.G. 24
Grom-Baumgarten, C. 29
Gunchick, C. 18

Habtesion, A. 23
Hackl, C. 33
Hahn, L. 28
Hammad, S. 23
Haueis, C. 3
Heeren, J. 30
Heine, M. 30
Heinsen-Groth, F. 21
Hellerbrand, C. 7, 8, 10, 22, 33, 36
Helmrich, N.L. 12
Henkel, J. 13
Hermanns, H.M. 16
Hirose, M. 39
Hov, J.R. 21
Hovorkova, A. 6
Huber, S. 3, 11, 25, 30, 37, 48
Hudel, H. 18
Huhn, A. 14

Ibrahim, S. 38, 39

Iftoda, O.M. 41
Irungbam, K. 15, 31

Jahn, D. 16
Jalan, R. 23

Kaiser, M. 45
Karampekos, G. 17
Karlsen, T.H. 21
Karsten, C.M. 35
Katsinelos, P. 19
Kemper, C. 35
Kempski, J. 11
Khomko, O. 26, 41, 42
Kircher, S. 16
Kispert, A. 45
Klotter, V. 18
Kolomojets, O. 42
Korotkyi, O.H. 1
Kountouras, J. 19
Krawczyk, M. 20
Kroy, D. 5, 46
Kummen, M. 21
Kuna, M. 13
Künstner, A. 27, 38, 39
Kushnir, O.V. 41

Lammert, F. 20
Lang, S. 33
Leder, D. 15
Lee, S. 29
Liwinski, T. 21
Lohse, A.W. 21
Lotter, H. 32
Lücke, J. 11
Lücke, K. 32
Lütjohann, D. 20

Mahli, A. 22, 33
Malek, L. 8
Manns, M.P. 45
Mantzaris, G.J. 17
Mantzoros, C.S. 19
Milkiewicz, P. 20
Mittrücker, H.-W. 32
Möbus, S. 45
Mohamed, F. 23
Müller, M. 32

Norman, G.L. 43
Nüssler, A.K. 29

Olde Damink, L. 29
Ostapchenko, L.I. 1
Ott, M. 45

Padem, S. 24
Pagel, R. 27, 38, 39
Papp, M. 43, 44
Pelczar, P. 25, 30
Plehutsa, O.M. 26, 40, 41, 42
Polymerou, V. 19
Polyzos, S.A. 19
Preisker, S. 35
Püschel, G. 13
Pylypenko, S.V. 1

Quack, T. 24

Ragab, M. 27
Rehman, A. 32
Rindtorff, N. 9
Roderfeld, M. 12, 14, 15, 18, 24, 28, 31
Roeb, E. 12, 14, 15, 18, 24, 28, 31
Rose-John, S. 32
Rühlemann, M. 21
Ruoß, M. 29
Rusak, O.B. 26

Sadik, C. 27
Sahin, H. 5
Said, M. 25
Schaltenberg, N. 30
Scheja, L. 30
Schippers, A. 5
Schmidt-Arras, D. 32
Schneider, F. 31
Schramm, C. 21
Schröder, D. 14
Schumacher, N. 32
Scognamilio, P. 30
Seitz, T. 22, 33
Sharma, A.D. 45
Shemerovskii, K.A. 34
Shiri, A.M. 11
Siemers, E. 18
Sina, C. 27, 35, 38, 39

Sipeki, N. 43
Skibbe, K. 35
Sommer, J. 7, 36
Steffens, N. 37
Steglich, B. 48
Sünderhauf, A. 27, 35, 38, 39
Sydorchuk, A.R. 26, 40, 41, 42
Sydorchuk, I.I. 26, 40, 41, 42
Sydorchuk, I.R. 26
Sydorchuk, L.P. 26, 40, 41, 42
Sydorchuk, R.I. 26, 40, 41, 42

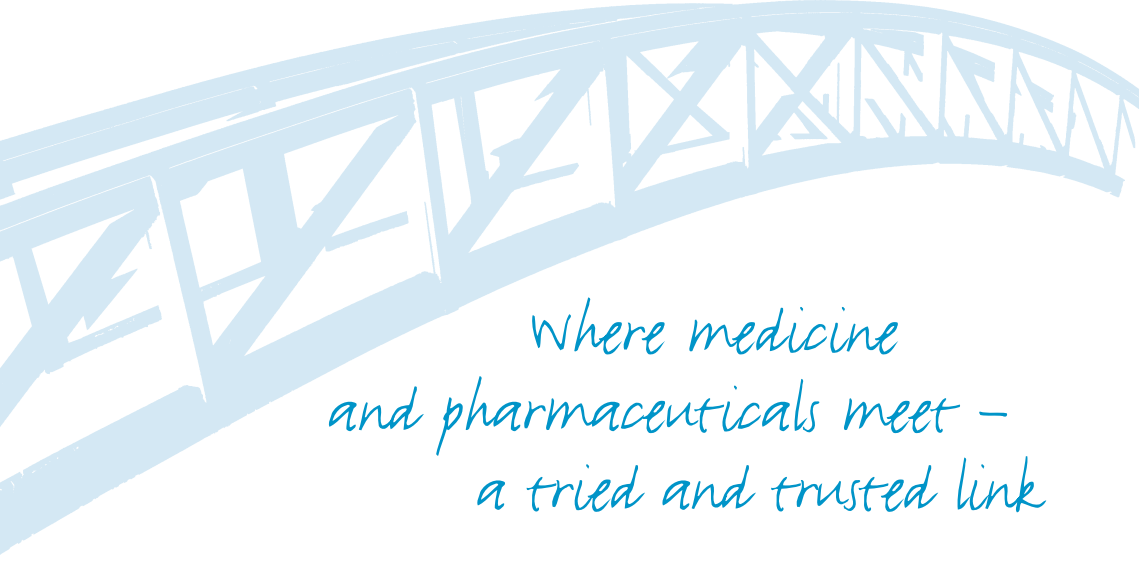
Tordai, A. 44
Tornai, D. 44
Tornai, I. 43, 44
Tornai, T. 43, 44
Trautwein, C. 5, 46
Tsay, H.-C. 45
Tschuschner, A. 12, 15, 24, 28

Van den Burg, L. 46
Vejrychova, J. 6
Verschoor, A. 35
Viazis, N. 17
Vitalis, Z. 43, 44
von Medem, K. 27

Wagner, A. 38
Wahib, R. 30
Wiese, M. 47
Wittek, A.F. 48
Worthmann, A. 30
Wunderlich, T. 32
Wunsch, E. 20

Yarynych, Y. 40
Yuan, Q. 45

Zazara, D. 11
Zenouzi, R. 21
Zhao, L. 11



Where medicine
and pharmaceuticals meet –
a tried and trusted link

FALK FOUNDATION e.V.

Leinenweberstr. 5
79108 Freiburg
Germany