to liver injury and fibrosis suggesting the beneficial role of intestinal microbiota in preventing liver injury[1, 2].

Disclosure of Interest: All authors have declared no conflicts of interest.

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OP352 IMPROVING METABOLIC PARAMETERS IN NAFLD BY TARGETING NUCLEAR RECEPTORS

P.M. Rodrigues[1], M.B. Afonso[1], A. Simão[1], M. Caridade[1], C. C. Carvalho[2], A. Trindade[2], A. Duarte[3], P. M. Borralho[1], M. V. Machado[4], H. Cortez-Pinto[5], C. M.P. Rodrigues[1], R. E. Castro[1]

[1]*Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon/Portugal*

[2]*Reproduction and Development, Interdisciplinary Centre of Research in Animal Health (CIISA), Faculty of Veterinary Medicine, Universidade de Lisboa, Lisbon/Portugal*

[3]*Gulbenkian Institute of Science, Oeiras/Portugal*

[4]*Gastrenterology, Hospital Santa Maria, Lisbon/Portugal*

[5]*Gastrenterology, Hospital Santa Maria, Lisbon/Portugal*

Contact E-mail Address: pmvrsrodrigues@ff.ul.pt

Introduction: Non-alcoholic fatty liver disease (NAFLD) pathogenesis and treatment remain unsolved. microRNAs and bile acids were recently suggested to participate in disease pathogenesis and, as such, constitute potential therapeutic tools and targets. Moreover, nuclear receptors, namely peroxisome proliferator-activated receptors (PPARs) and the farnesoid X receptor (FXR) are currently under scrutiny as modulators of lipid and glucose metabolism in non-alcoholic steatohepatitis (NASH).

Aims & Methods: We aimed to elucidate the role of the miR-21/PPARα pathway in liver and muscle tissues of murine NASH models and ascertain the therapeutic potential of miR-21 abrogation alone or in combination with obeticholic acid (OCA). Wild-type (WT) and miR-21 KO mice were fed with chow (n = 10) or methionine and choline-deficient (MCD; n = 10) diets for 2 and 8 weeks. Alternatively, mice were fed either chow (n = 12) or fast food diet (FF; n = 12) for 25 weeks. Six animals from each group had their diet supplemented with OCA 10 mg/kg/day (Intercept Pharmaceuticals, Inc.). Human liver biopsies were obtained from morbid obese NAFLD patients (n = 28). Liver/muscle samples were processed for histological analysis and assessment of miR-21, pro-inflammatory/pro-fibrogenic cytokines, PPARα and metabolic relevant genes, by qRT-PCR and immunoblotting. A Taqman® Array was performed to evaluate modulation of lipid regulated genes. ROS levels were analysed through the use of 2',7'- dichlorodihydrofluorescein diacetate.

Results: WT mice fed with the MCD diet developed steatohepatitis and fibrosis, displaying increased levels of apoptosis, necroptosis and serum ALT and AST. In contrast, miR-21 KO mice displayed a significant decrease in steatosis severity, liver damage, inflammation and did not develop fibrosis. WT FF-fed mice developed hepatomegaly, macrovesicular steatosis, inflammatory infiltrates and increased oxidative stress. miR-21 levels were increased in WT FF-fed mice, in both liver and muscle, concomitantly with decreased expression of PPARα, a key miR-21 target. Similar findings were observed in NAFLD patients. Further, WT FF+OCA-fed mice exhibited decreased steatosis and miR-21 expression, compared with WT FF-fed mice. Importantly, KO FF+OCA-fed mice exhibited significantly reduced liver inflammation, oxidative stress and steatosis, in parallel with increased expression of PPARα and its metabolic targets, including CPT-1 and ACOX2. Finally, lipid regulated genes such as ACAT1, ALOX5 and FABP5 were found to be severely deregulated in WT FF-fed mice and reverted to control levels in KO FF+OCA-fed mice.

Conclusion: In conclusion, activation of PPARα, as a result of miR-21 abrogation, together with FXR activation by OCA, significantly improves metabolic parameters in NASH, highlighting the therapeutic potential of multi-targeting therapies for NAFLD. (Supported by PTDC/BIM-MEC/0873/2012, SFRH/BD/88212/2012, FCT, Portugal).

Disclosure of Interest: All authors have declared no conflicts of interest.

WEDNESDAY, OCTOBER 19, 2016 08:30–10:00
MURINE MODELS OF INTESTINAL INFLAMMATION – ROOM 1.86

OP353 AN AUTOIMMUNITY-ASSOCIATED VARIANT IN PTPN22 PROTECTS FROM DISEASE ONSET IN MOUSE MODELS OF COLITIS

M. R. Spalinger[1], S. H. Kasper[1], C. Gottier[1], K. Atrott[1], S. Lang[2], G. Rogler[3], M. Scharl[4]

[1]*Gastroenterology And Hepatology, University Hospital Zurich, Zurich/Switzerland*

[2]*Division Of Gastroenterology And Hepatology, University Hospital Zurich, Zürich/Switzerland*

[3]*Klinik Für Gastroenterologie, UniversitätsSpital Zürich, Zürich/Switzerland*

[4]*Division Of Gastroenterology And Hepatology, University Hospital Zürich, Zürich/Switzerland*

Contact E-mail Address: Marianne.Spalinger@usz.ch

Introduction: Presence of the single nucleotide polymorphism (SNP) rs2476601 in the gene encoding protein tyrosine phosphatase non-receptor type 22 (PTPN22) results in an altered-function PTPN22 protein product and is associated with increased risk to develop autoimmune disorders, including type 1 diabetes, rheumatoid arthritis, systemic lupus erythematous. However, the same variant reduces the risk for Crohn's disease (CD) onset. We have previously shown that protein and mRNA levels of PTPN22 are reduced in intestinal biopsies from CD patients, and that loss of PTPN22 results in enhanced inflammatory cytokine secretion from mononuclear cells treated with interferon-gamma or the bacterial product muramyl dipeptide.

Aims & Methods: In this study, we addressed how presence of the altered-function variant in PTPN22 influences the susceptibility to intestinal inflammation in mouse models of colitis. For this aim, colitis was induced in 10–12 week old female mice by administration of 2% DSS for 7 days (acute DSS colitis), administration of four cycles of DSS (1.5% DSS for 7 days, followed by 10 days normal drinking water each; chronic DSS colitis), or by transferring naïve T cells into RAG2-/- recipients. PTPN22 deficient (PTPN22-/-) mice, or mice expressing the IBD-associated variant in PTPN22 (PTPN22-619 W mice), and their respective wild-type (WT) littermates were used for the study.

Result: PTPN22-/- mice suffered from aggravated acute DSS colitis as characterized by pronounced weight loss, increased endoscopic and histologic colitis scores ($p < 0.05$ each), while PTPN22-619 W mice reacted only weak to the DSS treatment when compared to WT littermates ($p < 0.05$ for weigh development, $p < 0.01$ for other parameters). In chronic DSS colitis however, PTPN22-/- mice suffered from a milder disease course (reduced weight loss [$p < 0.05$], decreased histological severity [$p < 0.05$]) from the third cycle onwards. PTPN22-619 W on the other hand mice tended to show a more pronounced disease course in the later phase. In the T cell transfer model, PTPN22-/- T cells induced an enhanced histological pathology ($p < 0.05$), while weight loss was not affected when compared to mice receiving WT T cells. In contrast, mice transfected with PTPN22-619 W T cells were protected from disease development in the first weeks, and later on developed only a mild disease (moderate weight loss [$p < 0.01$], reduced shortening of the colon [$p < 0.05$], low histological disease scores [$p < 0.05$]) when compared to mice receiving WT T cells.

Conclusion: Taken together, we here describe for the first time how the IBD-associated variant in PTPN22 affects colitis development. This helps to explain why this variant is associated with a reduced risk for CD onset, although it increases the risk to develop classical autoimmune disorders.

Disclosure of Interest: All authors have declared no conflicts of interest.

OP354 TOLL LIKE RECEPTOR 2 MODULATES THE INHIBITORY MOTOR RESPONSE INDUCED BY HYDROGEN SULPHIDE IN MOUSE COLON

R. Forcén García[1], E. Layunta[1], J. Pardo[2], J.E. Mesonero[1], L. Grasa[3]

[1]*Pharmacology And Physiology Department, University of Zaragoza, Zaragoza/Spain*

[2]*Biochemistry And Molecular And Cellular Biology Department, University of Zaragoza, Zaragoza/Spain*

[3]*Pharmacology And Physiology, Universidad de Zaragoza, Zaragoza/Spain*

Contact E-mail Address: r.forcen.90@gmail.com

Introduction: The recognition of intestinal microbiota is in part carried out by toll-like receptors (TLR), which are responsible for initiating the innate immune response. Alterations in the intestinal microbiota and its recognition may contribute to the development of intestinal inflammatory pathologies. Otherwise, hydrogen sulphide (H_2S) is an endogenous gaseous signalling molecule and it potentially plays a relevant role in the intestinal motility. In mammals, two pyridoxalphosphate-dependent enzymes are responsible for H_2S synthesis: cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE).

Aims & Methods: The aim of this study was to investigate the influence of TLR2 on the motor response induced by H_2S and the enzymes responsible for H_2S synthesis (CBS and CSE) in mouse colon. Colon strips from male C57/BL10 wild-type (WT) and TLR2$^{-/-}$ mice of 8–12 weeks old were suspended in an organ bath in the direction of circular smooth muscle. We studied the effect of NaHS (10 μM–1 mM), D,L-propargylglycine (PAG, 10 μm–10 mM), an inhibitor of CSE, and amino-oxyacetic acid (AOAA, 10 μm–10 mM), an inhibitor of CBS, on WT and TLR2$^{-/-}$ mice colonic motility. Gene expression (mRNA) of CSE and CBS were determined by real time-PCR and protein expression of CSE and CBS were quantified by Western blotting in colon from WT and TLR2$^{-/-}$ mice.

Results: The NaHS, as a source of exogenous H_2S, reduced the frequency but not the amplitude of the spontaneous contractions in colon from WT mice. The inhibition of CSE or CBS with PAG or AOAA, respectively, increased the frequency but not the amplitude of the spontaneous contractions in colon from WT mice. The NaHS induced a higher reduction of the frequency of the spontaneous contractions in TLR2$^{-/-}$ respect to WT mice. The PAG and AOAA did not modify the spontaneous contractions in colon from TLR2$^{-/-}$ mice. The mRNA and protein expression of CBS resulted decreased in colon of TLR2$^{-/-}$ compared with WT mice. The mRNA but not the protein expression of CSE resulted decreased in TLR2$^{-/-}$ compared with WT mice.

Conclusion: These results suggest that exogenous and endogenous H_2S may regulate the colonic spontaneous contractions in WT mouse, reinforcing the hypothesis that H_2S is a gaseous inhibitory mediator of intestinal motility. TLR2

regulates the expression of CBS and modulates the inhibitory motor response induced by H_2S in mouse colon.

Disclosure of Interest: All authors have declared no conflicts of interest.

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OP355 DIRECT INHIBITION OF HMGB1 BY NEUTRALIZING ANTIBODY AMELIORATES EXPERIMENTAL COLITIS IN MICE VIA TLR4-MYD88 PATHWAY

F. Xiao[1], B. Sun[1], J. Li[2], H. Mu[1], M. Zhang[1], D. Li[1], H. Huang[1], M. Liu[1], U. Seidler[3], D. Tian[1]

[1]*Gasroeterology Department, Huazhong Unversity Of Science And Technology Affiliated Tongji Hospital, Wuhan/China*

[2]*Huazhong Unversity Of Science And Technology Affiliated Tongji Hospital, Nephrology Department, Wuhan/China*

[3]*Gastroenterology Department, Hannover Medical School, Hannover/Germany*

Contact E-mail Address: christina_fx@126.com

Introduction: Biologics targeting inflammatory cytokines has reveal a new era in inflammatory bowel disease treatment. High mobility group protein B1 (HMGB1) acts as an alarmin in early stage and inflammatory cytokine in late stage during inflammation. Direct blockade of HMGB1 can be protective against intestinal inflammation.

Aims & Methods: Potential role of anti-HMGB1 neutralizing antibody (HnAb) in inhibiting intestinal inflammation and the underlying mechanism is investigated in DSS-induced mice colitis (DSS-C) models. 200μg HnAb was administrated intraperitoneally to DSS-C at d0, d3 and d6 in HnAb group, whereas 200μg anti-IgY was used as control in DSS-C (DSS-C group) or normal control (ctrl group). Colon shortening, disease activity index (DAI), histological score of colitis (HS), MPO activity and inflammatory cytokines were evaluated to determine the colonic inflammation severity. Mucosa barrier function was assessed by immunofluorescent staining of mucus layer (mucin2) and tight-junction (T-J) protein detection. mRNA was detected by qPCR. T-J protein, HMGB1, TLR4, MyD88 was detected by Western blotting and measured by Grey-scale value. Statistical analysis was performed using one-way ANOVA analysis and the Post Hoc LSD test or Tamhane's T2 test.

Results: Treatment with HnAb significantly suppressed colonic inflammation in DSS-C mice by improving colon shortening (6.2 ± 0.4 cm vs. 5.3 ± 0.5 cm, $p < 0.05$), DAI (2.7 ± 0.5 vs. 3.7 ± 0.3, $p < 0.05$) and HS (6.0 ± 0.1 vs. 9.6 ± 0.7, $p < 0.05$). Besides, MPO activity (2.6 ± 0.8 vs. 4.8 ± 1.0, $p < 0.05$) and TNF-α (1.61 ± 0.05 vs. 3.04 ± 0.11, $p < 0.05$), IFN-γ (2.14 ± 0.06 vs. 7.87 ± 0.21, $p < 0.05$) and IL-1β (1.53 ± 0.10 vs. 2.48 ± 0.04, $p < 0.05$) mRNA expression was decreased when treated with HnAb as compared to DSS-C group (mRNA in ctrl group was set to 1). Relatively intact mucus layer was seen in mice colon of HnAb group as compare to DSS-C group. Significantly higher expression of tight-junction protein ZO-1 (0.38 ± 0.01 vs. 0.15 ± 0.05, $p < 0.0001$), claudin-5 (0.50 ± 0.09 vs. 0.17 ± 0.07, $p < 0.0001$) and occludin (0.85 ± 0.09 vs. 0.39 ± 0.01, $p < 0.0001$) was detected in HnAb mice as compared to mice in DSS-C group. Interestingly, colonic HMGB1 protein in both nucleus (0.58 ± 0.02 vs. 0.79 ± 0.03, $p < 0.0001$) and cytoplasm (0.23 ± 0.01 vs. 0.40 ± 0.03, $p < 0.0001$) were all decreased when treated with HnAb as compare to DSS-C, suggesting that primary inhibition of HMGB1 by HnAb blocked sequential HMGB1 formation and release. Lastly, TLR4 (0.31 ± 0.03 vs. 0.77 ± 0.08, $p < 0.0001$) and MyD88 (0.30 ± 0.03 vs. 0.78 ± 0.01, $p < 0.0001$) protein was significantly reduced in HnAb group than mice in DSS-C group though MyD88 mRNA was relatively higher in HnAb group than DSS-C group (0.69 ± 0.04 vs. 0.38 ± 0.01, $p < 0.05$).

Conclusion: Administration of HnAb ameliorated DSS-C by suppressing inflammation and strengthening mucosa barrier function possibly through inhibition of HMGB1-TLR4-MyD88 pathway, suggesting a potential interventional target of HMGB1 in ulcerative colitis treatment.

Disclosure of Interest: All authors have declared no conflicts of interest.

OP356 NEW, PEPTIDE INHIBITOR OF DIPEPTIDYL PEPTIDASE IV, EMDB-1 ATTENUATES COLITIS IN MICE AFTER TOPICAL ADMINISTRATION

M. Salaga, P. Mosinska, H. Zatorski, M. Zielinska, J. Fichna

Dept. Of Biochemistry, Medical University of Lodz, Lodz/Poland

Contact E-mail Address: macieks100@wp.pl

Introduction: PETIR (PEptidase-Targeted ImunoRegulation) is a novel therapeutic strategy which takes for the purpose restoration of the immune balance by limiting the activation of immune cells and induction of endogenous protective mechanisms, such as TGFβ and glucagon-like peptide-2 (GLP-2) through inhibition of DPP IV-dependent pathways. Experimental data indicate that PETIR results in suppression of cell proliferation and reduced synthesis of pro-inflammatory cytokines without affecting cellular vitality.

Aims & Methods: The objective of this study was to test the anti-inflammatory activity of a novel DPP IV inhibitor EMDB-1 in the mouse models of colitis. The inhibitory effect of EMDB-1 on DPP IV was characterized in vitro using the HPLC system measuring the degradation rate of endomorphin-2 (EM2, natural DPP IV substrate) in the presence of the test compound. Anti-inflammatory activity of EMDB-1 was investigated in the model of acute and semi-chronic colitis induced by trinitrobenzenesulfonic acid (TNBS). Body weight, macroscopic score, ulcer score, colon length and thickness, as well as myeloperoxidase (MPO) activity were recorded. Mesalazine was used as a reference drug.

Results: EMDB-1 is a potent and specific DPP IV inhibitor as shown by significantly decreased degradation rate of EM2 by DPP IV ($t_{0.5} = 1.73$ vs. 3.60 min in the absence and the presence of EMDB-1, respectively). The intracolonic (i.c.) administration of EMDB-1 (0.1, 1 and 3 mg/kg, twice daily) attenuated both acute and semi-chronic TNBS-induced colitis in mice in a dose-dependent manner, as indicated by significantly reduced macroscopic parameters and MPO activity. Anti-inflammatory effect of EMDB-1 was not blocked by naloxone, thus the opioid receptors are not involved in its mechanism of action.

Conclusion: EMDB-1 is a potent inhibitor of DPP IV in vitro and exhibits substantial anti-inflammatory activity in the GI tract in vivo. Results of this study validate the EMDB-1 backbone for further development of peptide DPP IV inhibitors and suggest their potential use in the treatment of colitis.

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OP357 CHROMOFUNGIN (CHR) AMELIORATES EXPERIMENTAL COLITIS IN MICE VIA MODULATION OF MACROPHAGES' PLASTICITY

N. Eissa[1], F. Rabbi[1], M. Metz-Boutigue[2], C. Bernstein[3], J. Ghia[1]

[1]*Immunology & Internal Medicine, University of Manitoba, Winnipeg/Canada*

[2]*INSERM, Strasbourg/France*

[3]*Internal Medicine, Division of Gastroenterology, University of Manitoba, Winnipeg, Canada, Winnipeg/Canada*

Contact E-mail Address: Nour.Eissa@umanitoba.ca

Introduction: Macrophages play a major role in inflammatory bowel disease (IBD) pathogenesis through an inappropriate response to migration, and an impaired transition from a pro-inflammatory (classical activated macrophages (CAMs)) to an anti-inflammatory (alternative activated macrophages (AAMs)) phenotype. While there is growing awareness of a relationship between Chromogranin (Cg)-A and a susceptibility to inflammatory conditions, the specific interaction between CgA-derived peptides and macrophage plasticity in IBD is unknown. Recently, we have shown a linear correlation between CgA and inflammatory markers in patients with active ulcerative colitis, and colitic CgA-deficient mice demonstrated a significant decrease of colitis associated to a modulation of macrophage activation. As Cg-A is a prohormone, herein, we assessed the functional role of a specific CgA-derived peptides (Chromofungin (CHR): hCg-A47-66) in the regulation of acute colitis and the functional plasticity of murine macrophages.

Aims & Methods: Colitis was induced in C57BL/6 mice (7–8 weeks old) by administrating dextran sulfate sodium (DSS 5%) in drinking water for 5 days. Preventive CHR (2.5 mg/kg/day) or vehicle treatments started 1 day before induction of colitis and lasted for a total of 6 days. Disease activity index (DAI) was evaluated daily and mice were sacrificed on day 5 post-DSS induction to assess the extent of colitis. At sacrifice macroscopic scores were evaluated, serum level of C-reactive protein (CRP) was quantified using ELISA, and colonic interleukin (IL)-1β, IL-6, TNF-α, MIP-1α, MIP-1β, and ARG-1 were assessed using ELISA and RT-qPCR. Naïve peritoneal macrophages were isolated from non-colitic C57BL/6 mice and treated by CHR (200 ng/ml) then exposed for 6 h to LPS (100 ng/ml) to promote CAMs, or to IL-4/IL-13 (20 ng/ml) to promote AAMs. CAMs markers (IL-6, IL-1β, TNF-α, MIP-1α & MIP-β) and AAMs markers (ARG-1) were quantified by using ELISA and RT-qPCR.

Results: Preventive treatment with CHR significantly reduced the DAI onset and severity of colitis associated to rectal bleeding, stool consistency and weight loss. Macroscopic scores, serum-CRP, colonic IL-1β, IL-6, TNF-α, MIP-1α, MIP-1β were significantly decreased, while ARG-1 was significantly increased. In vitro, CHR-conditioned CAMS expressed significantly less IL-1β, IL-6, TNF-α, MIP-1α, MIP-β, but, surprisingly, more ARG-1 when compared to LPS control condition. Moreover, CHR-conditioned AMS expressed significantly more ARG-1 when compared to IL-4/IL-13 control condition.

Conclusion: These findings suggest that CHR can modulate the severity of experimental colitis. CHR treatment can attenuate the severity of experimental colitis and the inflammatory process via the modulation of the functional plasticity of murine macrophages and their functions. Targeting CgA-derived peptides may lead to novel therapeutic strategies in ulcerative colitis.

Disclosure of Interest: All authors have declared no conflicts of interest.

OP358 DEFICIENCY OF PH-SENSING RECEPTOR TDAG8 AMELIORATES T-CELL TRANSFER COLITIS

I. Tcymbarevich[1], C. De Vallière[1], J. Cosin-Roger[1], M. R. Spalinger[1], C. A. Wagner[2], K. Seuwen[3], I. Frey-Wagner[1], G. Rogler[4]

[1]*Gastroenterology And Hepatology, University Hospital Zurich, Zurich/Switzerland*

[2]*Institute Of Physiology, University of Zurich, Zurich/Switzerland*

[3]*Novartis Institutes for Biomedical Research, Basel/Switzerland*

[4]*Klinik Für Gastroenterologie, UniversitätsSpital Zürich, Zürich/Switzerland*

Contact E-mail Address: irina.tcymbarevich@usz.ch