Recovery of *Bacteroides thetaiotaomicron* ameliorates hepatic steatosis in experimental alcohol-related liver disease

Moris Sangineto[1,2], Christoph Grander[1], Felix Grabherr[1], Lisa Mayr[1], Barbara Enrich[1], Julian Schwärzler[1], Marcello Dallio[1,3], Antonio Moschetta[4], Timon E. Adolph[1], Carlo Sabbà[4], Gaetano Serviddio[2] & Herbert Tilg[1]

[1] Department of Internal Medicine I, Gastroenterology, Hepatology, Endocrinology & Metabolism, Medical University Innsbruck, Innsbruck, Austria

[2] C.U.R.E. (University Center for Liver Disease Research and Treatment), Liver Unit, Department of Medical and Surgical Sciences, University of Foggia, Foggia, Italy

[3] Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy

[4] Department of Interdisciplinary Medicine, University of Bari, Bari, Italy

Corresponding author: Herbert Tilg, Department of Internal Medicine I, Gastroenterology, Hepatology, Endocrinology & Metabolism, Medical University Innsbruck, Innsbruck, Austria.
Email: Herbert.Tilg@i-med.ac.at

Conflict of interest: None.

Number of figures: 6

Keywords: alcohol-related liver disease; Bacteroides thetaiotaomicron; microbiota; steatosis; intestinal barrier

Materials and methods

Expression studies

Tissue homogenization was performed with TRIzol® reagent (Thermo Fisher Scientific, Waltham, MA) to extract RNA. Reverse Transcription System (Thermo Fisher Scientific, Waltham, MA) was used for reverse transcription and consequently SybrGreen (Eurogentec, Seraing, Belgium) and Mx3000 Cycler (Stratagene California, CA) were used to perform qPCR. The gene expression was normalized to mouse β-actin.

Triglyceride analysis

Frozen (-80°C) liver tissue was homogenized in PBS, adjusting the volume to the weight, followed by 30 minutes incubation at 95° C. Sample was centrifugated at 12000g for 10 minutes at room temperature. Following, supernatant was harvested for triglycerides measurement using the appropriate reagent (Roche, Switzerland). Vials, used for the procedure, were previously coated with fatty-free BSA (Sigma, St. Louis, MO).

In vitro assay for evaluation of BT growth with ethanol

In order to investigate whether Bacteroides Thetaiotaomicron (BT) growth was affected by alcohol, LYBHI medium (37 g/L of brain-heart infusion (Sigma-Aldrich, St. Louis, MO) and 5 g/L of yeast extract (Conda)) was supplemented with ethanol (1%; 3%; 5%) and inoculated with BT. After 24h the number of bacteria in the medium was measured by CASY cell counter and analyser (OLS).

Mucus thickness and goblet cell counting.

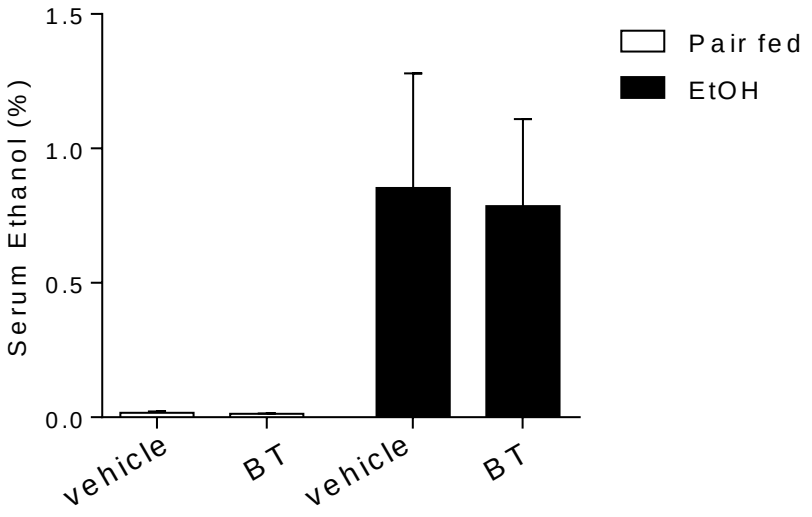
Colon tissue was fixed in Carnoy solution (6 parts of ethanol abs., 6 parts of acetic acid glacial, 1 part of chloroform) and followed by PAS staining.

The sections were analysed with Panoramic Viewer (3DHISTECH, Budapest, Hungary) to quantify mucus thickness (at least 60 measurements for each sample). The number of globet cells per colonic crypt and the mean dimeter were calculated analysing at least 12 crypts for each slide.

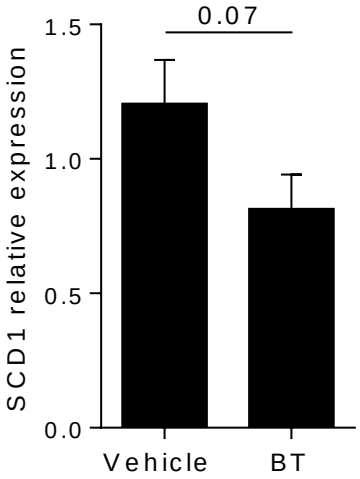
Histology

Liver and gut tissues were stained with haematoxylin and eosin (H&E) by the Institute of Pathology at the Medical University of Innsbruck.

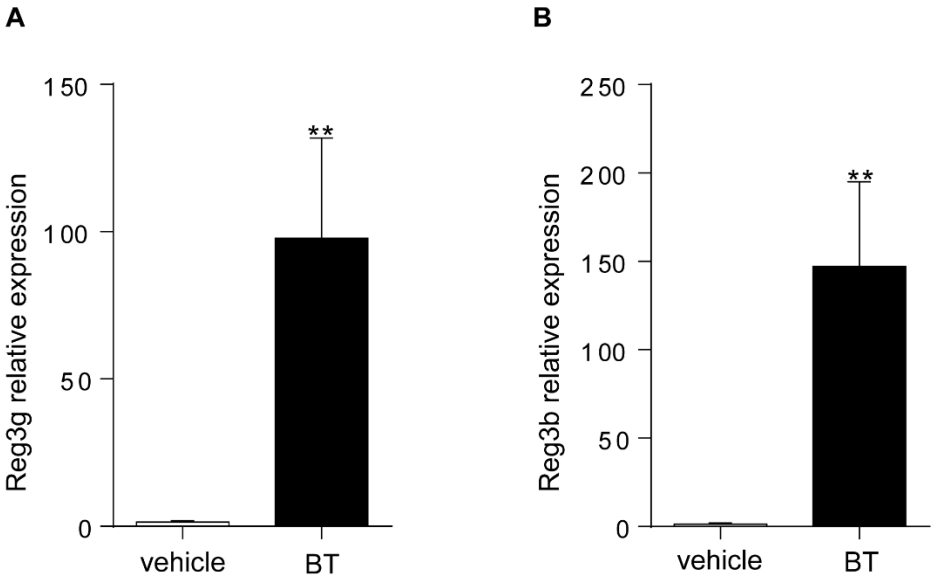
A pathologist analysed the h&e liver sections to evaluate hepatic steatosis in a blinded fashion, calculating the percentage of cells with lipid drops accumulation. The Myeloperoxidase immunohistochemistry on liver sections was performed as previously described(1). While, colon sections were used for immunofluorescence staining of Mucin-2 (Muc-2) as performed in a previous work (1). A 340 confocal microscope (Zeiss, Oberkochen, Germany) was used to analyse and take pictures of Muc-2 immunofluorescence.



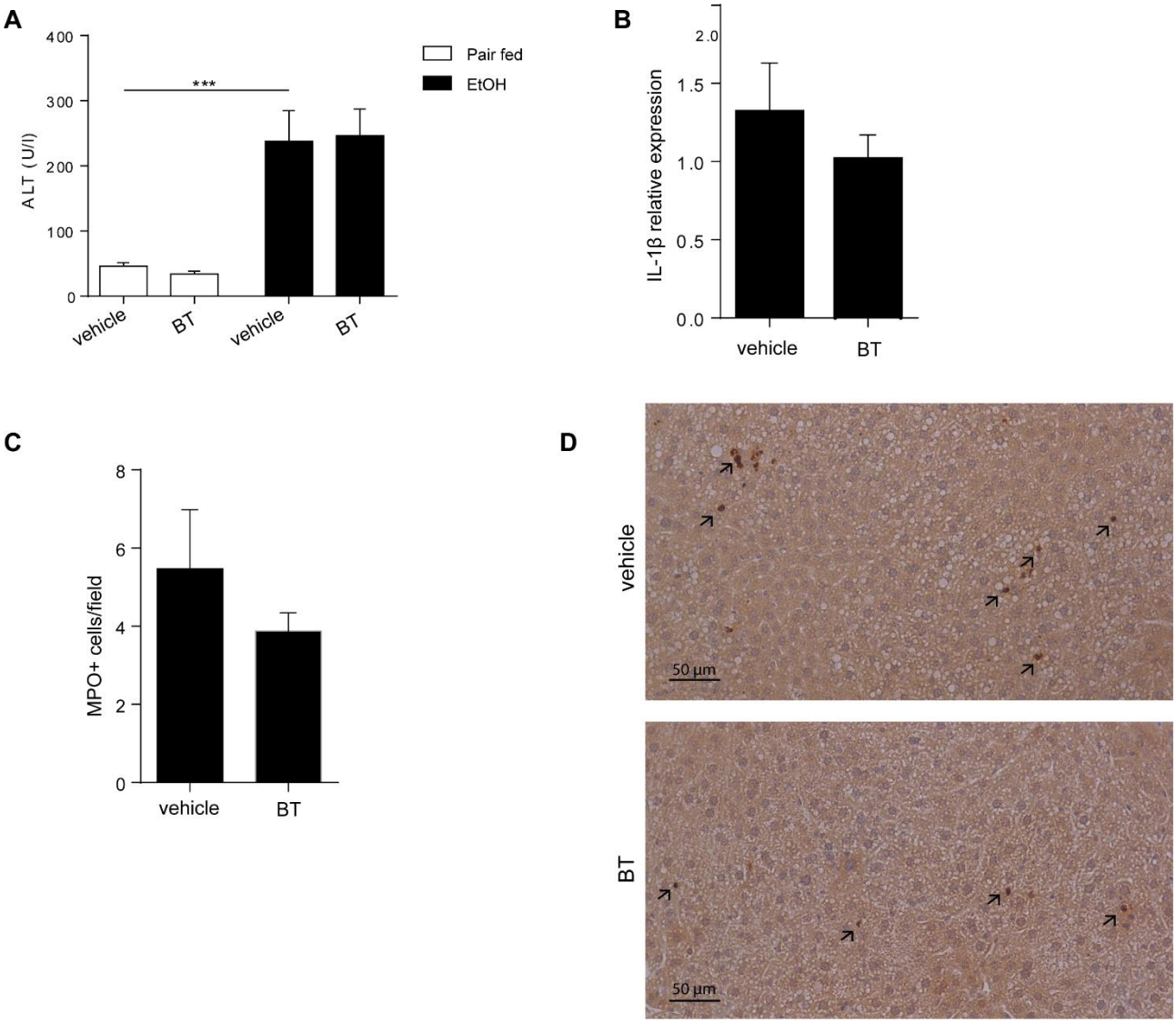
Supplementary Fig.1 vehicle and *Bt* treated mice assumed same quantity of alcohol. (A) Serum ethanol concentrations (Pair fed groups=n3; EtOH groups=n4-5). Data are expressed in mean ± SEM; *p<0.05 according to one-Way ANOVA followed by post hoc analysis (Bonferroni test)



Supplementary Fig.2 BT reduced hepatic SCD1 expression in EtOH fed mice. Hepatic expression of SCD1 in EtOH fed mice and determined by qPCR (vehicle=n8; BT=n10). Data are expressed in mean ± SEM; *p<0.05 according to two-tails student's t-test. SCD1, Stearoyl-CoA desaturase-1; BT, *Bacteroides Thetaiotaomicron*.



Supplementary Fig.3 BT up-regulates expression of antimicrobial peptides. (A, B) Intestinal expression of Reg3g and Reg3b in EtOH fed mice and determined by qPCR (n=8-10 per group). Data are expressed in mean ± SEM; *p<0.05; according to two-tails student's t-test. Reg3 -g -b, Regenerating islet-derived protein 3 -g -b; BT, *Bacteroides Thetaiotaomicron*.



Supplementary Fig.4 BT only midly reduced hepatic inflammation. (A) Serum ALT levels (Pair fed groups=n6; EtOH groups=n10). (B) Hepatic expression of IL-1β in EtOH fed mice (n=9-10 per group). (C, D) Representative pictures and quantification of neutrophils in liver tissue of EtOH fed mice, determined by immunoreactivity to MPO (brown; n=5 per group). Data are expressed in mean ± SEM; *p<0.05; **p<0.01; ***p<0.001, according to one-Way ANOVA followed by post hoc analysis (Bonferroni test) or two-tails student's t-test. BT, *Bacteroides Thetaiotaomicron*; MPO, myeloperoxidase.

Reference List

1. Grander C, Adolph TE, Wieser V, Lowe P, Wrzosek L, Gyongyosi B, et al. Recovery of ethanol-induced Akkermansia muciniphila depletion ameliorates alcoholic liver disease. Gut. 2018;67(5):891-901.