Original research

ATP citrate lyase (ACLY)-dependent immunometabolism in mucosal T cells drives experimental colitis in vivo

Anja Schulz-Kuhnt,[1,2] Katharina Rühle,[1] Asal Javidmehr,[1] Michael Döbrönti,[1] Jana Biwank,[1] Selina Knittel,[1] Peter Neidlinger,[1] Jannik Leupold,[1] Li-Juan Liu,[1] Mark Dedden,[1] Regina Verena Taudte,[3,4] Arne Gessner,[3] Martin F Fromm,[3] Dirk Mielenz,[5] Lucas Kreiss,[1,6] Maximilian J Waldner,[1] Sebastian Schürmann,[6] Oliver Friedrich,[6] Barbara Dietel,[7] Rocío López-Posadas ,[1,8] Christina Plattner,[9] The TRR241 IBDome Consortium,[10] Sebastian Zundler ,[1] Christoph Becker ,[1] Raja Atreya ,[1] Markus F Neurath ,[1,8] Imke Atreya [1,8]

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/gutjnl-2023-330543).

For numbered affiliations see end of article.

Correspondence to
Dr Imke Atreya, Department of Medicine 1, University Hospital of Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Bayern, Germany;
imke.atreya@uk-erlangen.de

MFN and IA contributed equally.

MFN and IA are joint senior authors.

Received 22 June 2023
Accepted 12 December 2023
Published Online First
4 January 2024

Check for updates

© Author(s) (or their employer(s)) 2024. No commercial re-use. See rights and permissions. Published by BMJ.

To cite: Schulz-Kuhnt A, Rühle K, Javidmehr A, *et al*. *Gut* 2024;**73**:601–612.

ABSTRACT

Objective Mucosal T cells play a major role in inflammatory bowel disease (IBD). However, their immunometabolism during intestinal inflammation is poorly understood. Due to its impact on cellular metabolism and proinflammatory immune cell function, we here focus on the enzyme ATP citrate lyase (ACLY) in mucosal T cell immunometabolism and its relevance for IBD.

Design ACLY expression and its immunometabolic impact on colitogenic T cell function were analysed in mucosal T cells from patients with IBD and in two experimental colitis models.

Results ACLY was markedly expressed in colon tissue under steady-state conditions but was significantly downregulated in lamina propria mononuclear cells in experimental dextran sodium sulfate-induced colitis and in $CD4^+$ and to a lesser extent in $CD8^+$ T cells infiltrating the inflamed gut in patients with IBD. ACLY-deficient $CD4^+$ T cells showed an impaired capacity to induce intestinal inflammation in a transfer colitis model as compared with wild-type T cells. Assessment of T cell immunometabolism revealed that ACLY deficiency dampened the production of IBD-relevant cytokines and impaired glycolytic ATP production but enriched metabolites involved in the biosynthesis of phospholipids and phosphatidylcholine. Interestingly, the short-chain fatty acid butyrate was identified as a potent suppressor of ACLY expression in T cells, while IL-36α and resolvin E1 induced ACLY levels. In a translational approach, in vivo administration of the butyrate prodrug tributyrin downregulated mucosal infiltration of $ACLY^{high}$ $CD4^+$ T cells and ameliorated chronic colitis.

Conclusion ACLY controls mucosal T cell immunometabolism and experimental colitis. Therapeutic modulation of ACLY expression in T cells emerges as a novel strategy to promote the resolution of intestinal inflammation.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ A relevant number of patients with inflammatory bowel disease (IBD) do not achieve a complete and/or sustained response to currently available therapeutic strategies.
⇒ The currently established concept in IBD therapy is to interfere with cytokine-driven immune cell activation and differentiation or integrin-dependent and chemokine-dependent immune cell gut homing, while the aspect of immunometabolism at the interface between mucosal immune cells, gut microbiota and absorbed nutrients received less attention, although potentially offering a broad spectrum of new therapeutic targets.
⇒ The enzyme ACLY crucially links cell metabolism with immune cell function by catalysing the cytoplasmatic formation of acetyl coenzyme A and thereby impacting on the differentiation and cytokine profile of T cells.

WHAT THIS STUDY ADDS

⇒ This is the first study describing the regulation of ACLY protein expression in intestinal $CD4^+$ T cells of patients with IBD and defining the functional in vivo role of this metabolic enzyme in T cell-driven chronic colitis.
⇒ Our data strongly suggest that the inflammation-associated downregulation of ACLY in lamina propria T cells supports the resolution of intestinal inflammation.
⇒ Regarding the therapeutic targetability of ACLY, the short-chain fatty acid (SCFA) butyrate was identified as a potent suppressor of ACLY expression in T cells.

INTRODUCTION

Inflammatory bowel disease (IBD) therapy has been revolutionised in the last three decades by the very successful introduction of new biological agents (e.g., anti-TNF therapy, the anti-α4β7 integrin antibody vedolizumab and the IL-12/IL-23-neutralising antibody ustekinumab) specifically interfering with the underlying immunopathogenesis. However,

Gut: first published as 10.1136/gutjnl-2023-330543 on 4 January 2024. Downloaded from http://gut.bmj.com/ on March 18, 2024 at Universitatsbibliothek Erlangen Nurnberg. Protected by copyright.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ By demonstrating the capacity of the SCFA-based ester tributyrin to ameliorate chronic colitis, we provide a concrete strategy for targeting the immunometabolic effects of ACLY in IBD therapy.

approximately 40% of patients with IBD do not achieve a satisfactory and long-lasting response to these currently available therapeutic regimens.[1 2] Thus, aiming to minimise the frequency of non-responding patients, it seems attractive to set out for new shores and consider a widened spectrum of immune cell-modulating factors of relevance in intestinal inflammation that can serve as therapeutic target. While recent efforts mainly focused on blocking cytokine-driven T lymphocyte activation or integrin-dependent and chemokine-dependent T cell gut homing,[3] the aspect of T cell immunometabolism has received less attention. This is particularly surprising when considering the proven relevance of immunometabolism at the interface between mucosal immune cells, gut microbiota and absorbed nutrients for the intestinal immunohomoeostasis and for the function of $CD4^+$ T lymphocytes as important key players of IBD pathogenesis.[4–6] Indeed, several studies indicated that the metabolic status of immune cells influences their effector function and colitis-promoting capacity, while components of the inflammatory microenvironment and microbial metabolites can shape the metabolic behaviour of mucosal immune cells.[7] In particular, the concept of metabolic reprogramming is well established, meaning that activated immune cells upregulate aerobic glycolysis at the expense of oxidative phosphorylation and thereby adapt their metabolic cellular reactions to the biosynthetic and energy requirements defined by the inflammatory context.[8] In IBD, tissue damage and loss of epithelial integrity in the chronically inflamed gut cause an increased mucosal incorporation of luminal bacteria and their metabolites,[9] which can, subsequently, influence the capacity of lamina propria (LP) immune cells to further trigger or, in contrast, resolve the inflammatory process.[10] The molecular pathways involved in this milieu-dependent metabolic rearrangement in T cells can offer new therapeutic targets, which would have a great potential to valuably complement already existing therapies for the control of the overwhelming intestinal immune response in patients with IBD.[7 8]

By catalysing the formation of cytosolic acetyl coenzyme A (AcCoA) from mitochondria-released citrate, the metabolic enzyme ATP citrate lyase (ACLY) not only forms a molecular link between cellular glucose metabolism and de novo lipogenesis but also provides acetyl groups for the epigenetic mechanism of histone acetylation and, thus, represents a key player of cellular metabolism.[11] Several studies already suggested an influence of ACLY-catalysed AcCoA generation on the expansion and effector function of $CD4^+$ T helper cells and $CD8^+$ T cells, which seems to be mediated via the modulation of epigenetic gene regulation and/or alterations in the de novo fatty acid synthesis required for biomass increase of proliferating cells.[11–14] For instance, ACLY expression was found to be upregulated in $CD4^+$ T cells cultured under Th17 conditions, while exposure of a leukaemia T cell line to IL-2 triggered phosphorylation and thus presumably activation of ACLY.[11 15 16] In contrast, declined ACLY activity was described in TGF-β-induced regulatory T cells (T_{reg} cells).[17] Accordingly, on a functional level, ACLY has been identified as modulator of Th17 effector molecules and of IFNγ expression in Th1 cells and $CD8^+$ T cells[11–13] and as regulator of T_{reg} induction,[17] thus impacting on key aspects of IBD pathogenesis.[18 19] Although there is growing evidence for a relevant involvement of ACLY in the modulation of T cell-driven inflammatory and malignant diseases,[11 13 20] the functional role of this metabolic enzyme in chronic colitis and IBD remains incompletely defined.

Addressing this gap in knowledge, we here defined the expression profile of ACLY and its inflammation-dependent regulation in intestinal T cells in colitis and IBD. Our study described for the first time a relevant functional impact of ACLY on the metabolic profile and colitogenic capacity of gut-infiltrating effector $CD4^+$ T cells in vivo. Finally, the short-chain fatty acid (SCFA)-based ester tributyrin was identified and in vivo validated as an ACLY-modulating drug for potential therapeutic intervention.

METHODS

Methods are available as online supplemental file.

RESULTS

Inflammation-associated downregulation of ACLY in intestinal T cells in IBD

To determine the relevance of ACLY in intestinal immune cells under homoeostatic and inflammatory conditions, we first analysed the protein expression levels of this metabolic enzyme in colon tissue lysates or purified LP mononuclear cells (LPMCs) and intestinal epithelial cells (IECs) derived from healthy control mice or mice previously exposed to the epithelium-damaging and colitis-inducing substance dextran sodium sulfate (DSS). While ACLY expression was clearly detectable in LPMCs and IECs under steady-state conditions, we observed significantly reduced levels of intestinal ACLY protein expression in DSS colitis. The inflammation-associated downregulation of ACLY could be observed in lysates of complete colon tissue and, even more pronounced, in the T cell-enriched fraction of LPMCs (figure 1A). Interestingly, the level of ACLY downregulation in colon tissue and purified LPMCs appeared to be dependent on the severity of colitis, reaching the highest extent in those mice showing high endoscopic colitis scores (online supplemental figure 1). In contrast to the colitis-associated decrease of ACLY protein levels in the local colonic immune cell compartment, this observation was less evident and did not reach statistical significance in IECs and was not detectable in peripheral immune cells in the spleen (figure 1A), implicating that the metabolic involvement of ACLY in LPMCs is regulated by the local inflammatory microenvironment. In accordance with the observed downregulation of ACLY expression in intestinal immune cells during experimental murine colitis, confocal immunofluorescence microscopy of human tissue samples derived from inflamed and non-inflamed gut areas of patients with IBD (figure 1B) and Western blot analyses of purified human IBD LPMCs and IECs (figure 1C) confirmed the inflammation-associated decrease of ACLY expression in intestinal LPMCs and in particular in colon-infiltrating $CD4^+$ T cells for the human disease. This phenomenon could be observed in a comparable extent in patients diagnosed for Crohn's disease or ulcerative colitis and appeared to be independent from the localisation of the inflammation in small or large intestine (online supplemental figure 2A), but was more pronounced in LP $CD4^+$ T cells than in the fraction of LP $CD8^+$ T cells (figure 1D–E). While the protein expression of ACLY was comparable between $CD4^+$ T cells and $CD8^+$ T cells in the peripheral blood and in non-inflamed gut tissue of individual patients with IBD, $CD4^+$ T cells in the inflamed gut tissue showed a significantly lower ACLY protein expression

Gut: first published as 10.1136/gutjnl-2023-330543 on 4 January 2024. Downloaded from http://gut.bmj.com/ on March 18, 2024 at Universitatsbibliothek Erlangen Nurnberg. Protected by copyright.

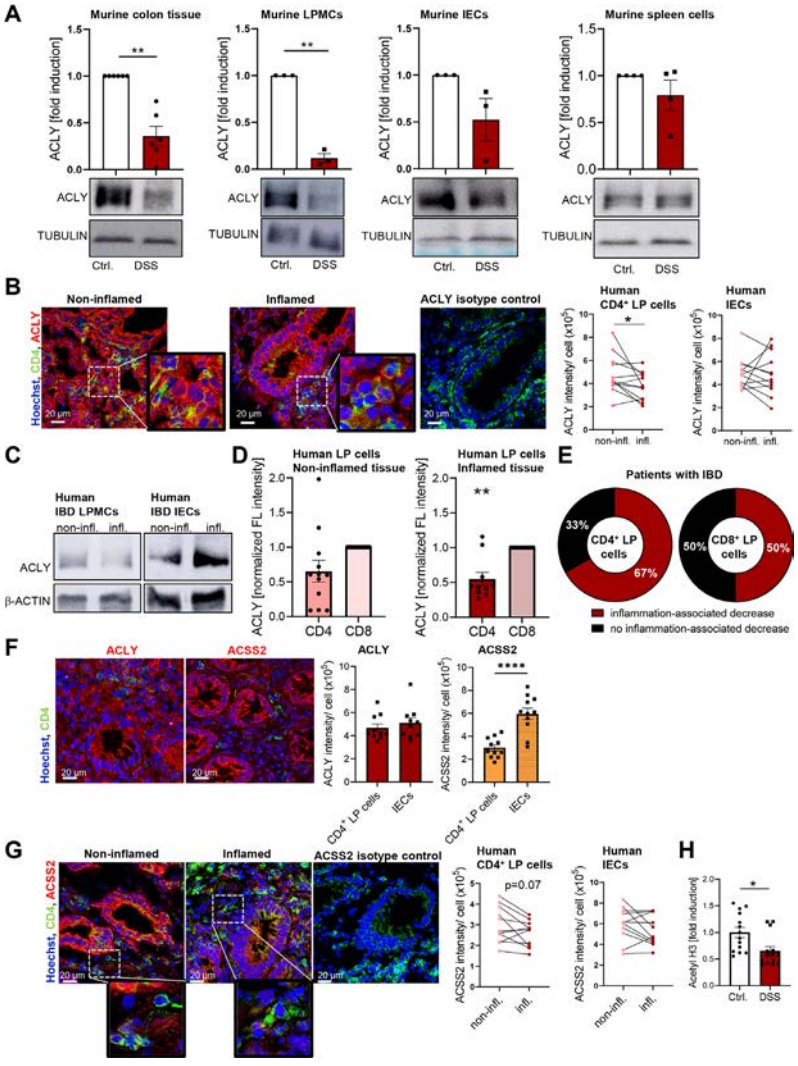

Figure 1 Downregulation of ACLY expression in intestinal T cells in the context of colitis. (A) ACLY protein expression in colon tissue, LPMCs, IECs and spleen cells of DSS-exposed mice was analysed via Western blot. Graphs summarising data from at least n=3 mice plus representative images are shown. (B) Intensity of the ACLY expression in CD4$^+$ T cells was quantified and compared between non-inflamed and inflamed gut mucosa of n=12 patients with IBD (n=7 patients with Crohn's disease; n=5 patients with ulcerative colitis) based on immunofluorescence staining of ACLY (RED) and CD4 (GREEN) in cryo-conserved intestinal tissue slides, followed by confocal microscopy and software (Fiji)-supported quantification of the ACLY-signal intensity detected in CD4$^+$ T cells and IECs. Representative images (including also isotype control staining) plus summarising graphs depicting the average ACLY intensity per CD4$^+$ cell or IEC (linked single data points for each individual patient) are shown. (C) ACLY protein expression (Western blot) in corresponding human LPMCs and IECs purified from macroscopically non-inflamed and inflamed gut tissue of a patient with IBD. (D) ACLY protein expression was analysed via flow cytometry in LPMCs purified from n=12 biopsies of endoscopically non-inflamed IBD gut tissue and from n=11 biopsies of endoscopically inflamed IBD gut tissue. The normalised ACLY fluorescence intensity was compared between LP CD4$^+$ T cells and LP CD8$^+$ T cells for each individual patient. (E) Based on intracellular immunofluorescence staining of ACLY and subsequent flow cytometric analyses, intensity of ACLY expression in CD4$^+$ T cells (n=6) and CD8$^+$ T cells (n=8) was quantified (mean fluorescence (FL) intensity) and compared between LPMCs purified from non-inflamed and inflamed gut mucosa of individual patients with IBD. Only analyses with an ACLY mean FL intensity >1 under non-inflamed conditions were included. Pie charts summarising the percentage of patients with IBD with an inflammation-associated downregulation of ACLY protein expression in CD4$^+$ T cells and CD8$^+$ T cells are shown. (F) ACLY (RED) signal intensity and ACSS2 (RED) signal intensity in CD4$^+$ T cells (GREEN) and IECs was quantified via immunofluorescence microscopy and compared for both cell types in non-inflamed tissue of n=10–11 patients with IBD (n=6–7 patients with Crohn's disease; n=4 patients with ulcerative colitis). Representative images and summarising graphs (single data points and mean±SEM) are depicted. (G) The immunofluorescence microscopically detected and quantified ACSS2 signal intensity (RED) in LP CD4$^+$ T cells (GREEN) and IECs was compared between non-inflamed and inflamed gut areas of n=11 patients with IBD (n=7 patients with Crohn's disease; n=4 patients with ulcerative colitis). Representative images (including also isotype control staining) and summarising graphs (linked single data points for each individual patient) are depicted. (H) Acetyl-Histone H3 expression in colon tissue of naïve versus DSS-exposed mice was analysed via Western blot. Signal density was quantified (Fiji software), whereby the expression of total histone H3 served as reference control. The summarising graph (single data points and mean±SEM) is based on data acquired in 6 experiments including in total 14 animals per group. Data from each experiment have been normalised to the mean value of naïve mice. Single outliers were identified using the Grubbs test and excluded from further analysis in (B, F). Ratio-paired t-test (B, G), paired t-test (F) or unpaired t-test (H) was applied. For relative data (A, D) the one-sample t-test was used. *p < 0.05, **p < 0.01, ****p < 0.0001. ACSS2, acyl-coenzyme A synthetase short-chain family member 2; DSS, dextran sodium sulfate; IBD, inflammatory bowel disease; IECs, intestinal epithelial cells; LPMCs, lamina propria mononuclear cells.

Gut: first published as 10.1136/gutjnl-2023-330543 on 4 January 2024. Downloaded from http://gut.bmj.com/ on March 18, 2024 at Universitatsbibliothek Erlangen Nurnberg. Protected by copyright.

than the CD8$^+$ T cells that were located here (figure 1D, online supplemental figure 2B). Also, in good accordance with the findings collected in the experimental murine colitis model, the inflammation-associated downregulation of ACLY protein expression was restricted to locally accumulating intestinal T cells and could not be observed in peripheral blood-derived T cells of patients with IBD (online supplemental figure 2C). Interestingly, the observed regulation of ACLY protein levels during colitis apparently occurred on a post-translational level, as *Acly*/*ACLY* mRNA expression remained stable in LPMCs purified from mice with DSS-induced colitis compared with control animals (online supplemental figure 2D), was not regulated in the inflamed colon tissue of patients with IBD (online supplemental figure 2E) and neither correlated with the severity of intestinal inflammation (based on histology score; Modified Riley Score[21]) in patients with ulcerative colitis (online supplemental figure 2F).

As ACLY is responsible for generating extramitochondrial AcCoA, its inflammation-associated downregulation can be expected to relevantly reduce the availability of acetyl groups in LP CD4$^+$ T cells and, thereby, impact on epigenetic gene regulation via histone acetylation. However, it is well established that under specific conditions the lack of ACLY can be compensated, at least partly, by another metabolic enzyme, namely acyl-coenzyme A synthetase short-chain family member 2 (ACSS2). ACSS2 uses exogenous acetate to generate cytosolic AcCoA independently of ACLY.[22] To estimate the relevance of ACSS2 in intestinal immune cells and its potential compensatory upregulation in ACLY^{low} LP T cells during inflammation, we analysed the ACSS2 protein expression in LP CD4$^+$ T cells and IECs of patients with IBD. In contrast to the expression pattern of ACLY, ACSS2 protein was predominantly detected in the intestinal epithelium under steady-state conditions (figure 1F), which was in good accordance with the single-cell transcriptome in human colon biopsies as evaluated in a publicly available dataset (GSE116222)[23] based on the analyses of human colon biopsies (online supplemental figure 2G). Moreover, ACSS2 protein was not induced in CD4$^+$ T cells infiltrating the inflamed intestine of patients with IBD compared with corresponding non-inflamed control tissue (figure 1G). Thus, a relevant ACSS2-mediated functional compensation of the inflammation-associated ACLY decrease in IBD LP CD4$^+$ T cells cannot be expected. Two recent studies interestingly introduced mitochondria-derived or peroxisome-derived acetyl-carnitine as another alternative source for the ACLY-independent and ACSS2-independent generation of cytoplasmatic AcCoA.[24 25] However, as Western blot experiments showed significantly diminished levels of histone acetylation in the inflamed colon tissue of DSS-exposed mice (figure 1H), our data imply that alternative metabolic routes cannot fully compensate for the inflammation-associated downregulation of ACLY in the intestinal mucosa.

Our data on ACLY expression in IBD and experimental colitis thus strongly implicate an inflammation-triggered and functionally relevant reduction of ACLY activity in locally accumulating intestinal CD4$^+$ T cells.

ACLY impacts on the immunometabolism and the colitis-inducing capacity of CD4$^+$ T cells

The observed regulation of ACLY expression in intestinal T cells during inflammation further increased our interest in elucidating the functional influence of ACLY on the proinflammatory capacity of T lymphocytes as key drivers of IBD pathogenesis.[26] In order to focus on this aspect, we took advantage of conditional knockout mice carrying a T cell-restricted ACLY deficiency (ACLY$^{\Delta CD4}$ mice) (online supplemental figure 3A) and the experimental model of adoptive transfer colitis. In this chronic colitis model, adoptively transferred naïve CD4$^+$CD25^{neg}CD44^{low} T cells accumulate in the intestinal mucosa of immunodeficient *Rag1* KO recipient mice and drive the development of colitis.[27] In accordance with the initial hypothesis that ACLY activity might be of key relevance for the behaviour of mucosal T cells, the transfer of ACLY-deficient naïve CD4$^+$ T cells into *Rag1* KO mice resulted in a significantly decreased level of intestinal inflammation compared with control mice transferred with ACLY-proficient CD4$^+$ T cells (figure 2A–D). In line with the clinical phenotype, the mRNA expression levels of the inflammation-indicating cytokine *Il-1b* was markedly reduced in the colon tissue of recipient mice transferred with ACLY-deficient CD4$^+$ T cells as compared with wild-type T cells (figure 2E). In contrast to the observed dependency of T cell-driven chronic experimental colitis on the presence of ACLY in CD4$^+$ T cells, the lack of the enzyme did not impact on the development of acute chemically induced colitis that is not critically dependent on T cells.[27] Analysing ACLY$^{\Delta CD4}$ mice in the acute model of DSS-induced colitis, we could not observe signs of decreased inflammatory gut pathology compared with control mice carrying ACLY-proficient T cells (online supplemental figure 3B), implicating that the level of ACLY activity in colitis-driving T cells is of particular functional relevance for the immunopathogenesis of chronic T cell-dependent colitis.

Although the milder intestinal inflammation in the recipient mice transferred with ACLY-deficient CD4$^+$ T cells in the adoptive transfer colitis model went along with a significantly reduced number of gut-infiltrating T cells (figure 2F), neither the viability and activation status of locally accumulating intestinal T cells nor the frequency of FoxP3$^+$ T$_{reg}$ cells in the intestinal immune cell compartment or the gut homing receptor expression of T cells in peripheral blood were influenced by the absence of ACLY (figure 2G–H). Also the immunological steady-state phenotype of ACLY$^{\Delta CD4}$ mice made it unlikely that the targeted deletion of ACLY is incompatible with T cell survival or gut homing of systemic T cells. The recently described observation that frequencies of peripheral T cells were unaltered in ACLY$^{\Delta CD4}$ mice[13] was here confirmed for LPMCs (online supplemental figure 3C). Moreover, also under steady-state conditions ACLY-deficient CD4$^+$ T cells showed an unaltered surface expression of the gut homing markers integrin α4 and β7 (online supplemental figure 3D), demonstrated an unaltered adhesion to MadCAM-1 in dynamic adhesion assays (online supplemental figure 3E) and did not show an increased induction of apoptosis or decreased survival on in vitro stimulation (online supplemental figures 3F–G). Altogether, our data point to a more specific function of ACLY in the immunometabolic regulation of intestinal T cell homoeostasis.

A subsequently performed immunometabolic characterisation based on mRNA analyses and Seahorse technology of the purified T cell-enriched LPMC fraction of mice previously transferred with ACLY-deficient T cells indicated a significant downregulation of the IBD-relevant pro-inflammatory cytokines *Tnfa*, *Il-17a, Ifng* and *Il-13* on in vitro stimulation, while other T cell-derived cytokines, like *Il-2* and *Il-3*, remained unaffected (figure 3A). This implicated a specific relevance of ACLY for the induction of proinflammatory T effector cell function in the intestinal mucosa. In accordance with the established concept that the induction of proinflammatory effector cell function goes along with metabolic reprogramming,[8] the purified ACLY-deficient LPMCs did not only show an impaired capacity to

Gut: first published as 10.1136/gutjnl-2023-330543 on 4 January 2024. Downloaded from http://gut.bmj.com/ on March 18, 2024 at Universitatsbibliothek Erlangen Nurnberg. Protected by copyright.

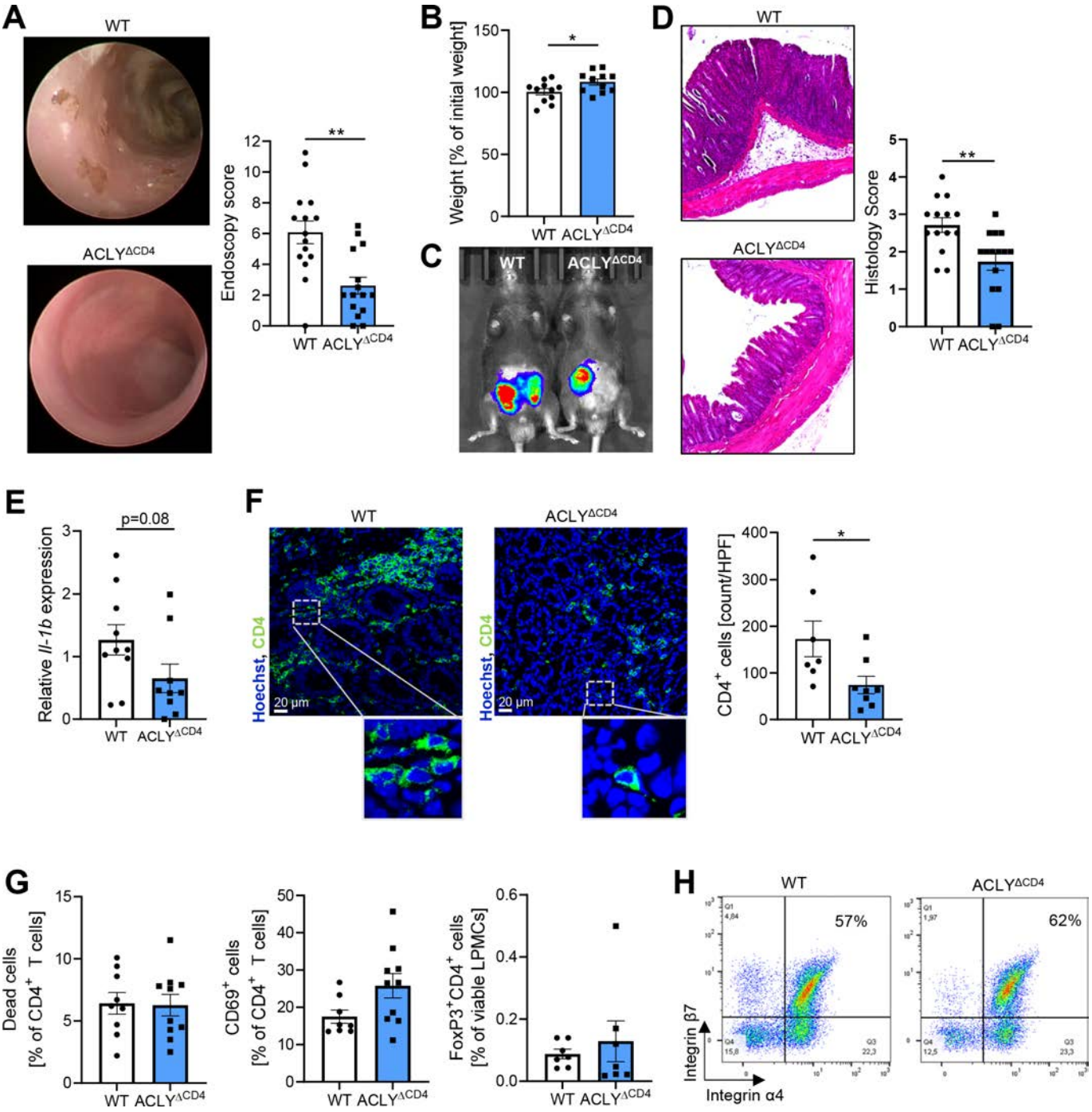

Figure 2 Decreased colitis-inducing capacity of ACLY-deficient CD4$^+$ T cells. Experimental colitis was induced by adoptive transfer of ACLY-proficient or ACLY-deficient CD4$^+$CD25$^-$CD44^{low} T cells into *Rag1* KO mice. (A) Colitis severity was monitored endoscopically at the end of the experiment (n=15 per group) and (B) based on body weight changes at weeks 5–6 after cell transfer (n=11 per group). Representative endoscopic images plus summarising graphs (single data points and mean±SEM). In addition, (C) in vivo IVIS luminescence images of reactive oxygen species after intraperitoneal injection of L-012 at the end of the experiment (n=8 analysed mice per group; representative images) and (D) histological ex vivo analyses of the colon tissue (n=14–15 per group) are shown. (E) At the end of the experiment, colonic tissue was harvested and analysed for *Il-1b* mRNA expression via qPCR. *Hprt* served as reference gene. Summarising graph is shown (single data points and mean±SEM of n=9–10 animals/group; data were normalised based on the mean ΔCT value of the control group). (F) Colonic infiltration of CD4$^+$ T cells was quantified ex vivo via immunofluorescence microscopy. Representative images and a summarising graph depicting single data points and mean±SEM of n=7–8 animals/group are shown. (G) LPMCs were purified from colon tissue of recipient mice and the following cell frequencies were quantified via flow cytometry (single data points and mean±SEM of n=7–10 per group): dead cells on CD4$^+$ lymphoid LPMCs; activated CD69$^+$ cells on CD4$^+$ lymphoid LPMCs; and FoxP3$^+$ CD4$^+$ cells on viable LPMCs. (H) Moreover, peripheral blood cells of a recipient mouse per group were flow cytometrically analysed for the expression of the gut homing receptors integrin α4 and integrin β7 on CD3$^+$CD4$^+$ T cells; dot plots are shown. Single outliers were identified using the Grubbs test and excluded from further analysis. Mann-Whitney U test (A) or unpaired t-test (B, D, E–G) was applied. *p < 0.05, **p < 0.01. LPMCs, lamina propria mononuclear cells.

Gut: first published as 10.1136/gutjnl-2023-330543 on 4 January 2024. Downloaded from http://gut.bmj.com/ on March 18, 2024 at Universitatsbibliothek Erlangen Nurnberg. Protected by copyright.

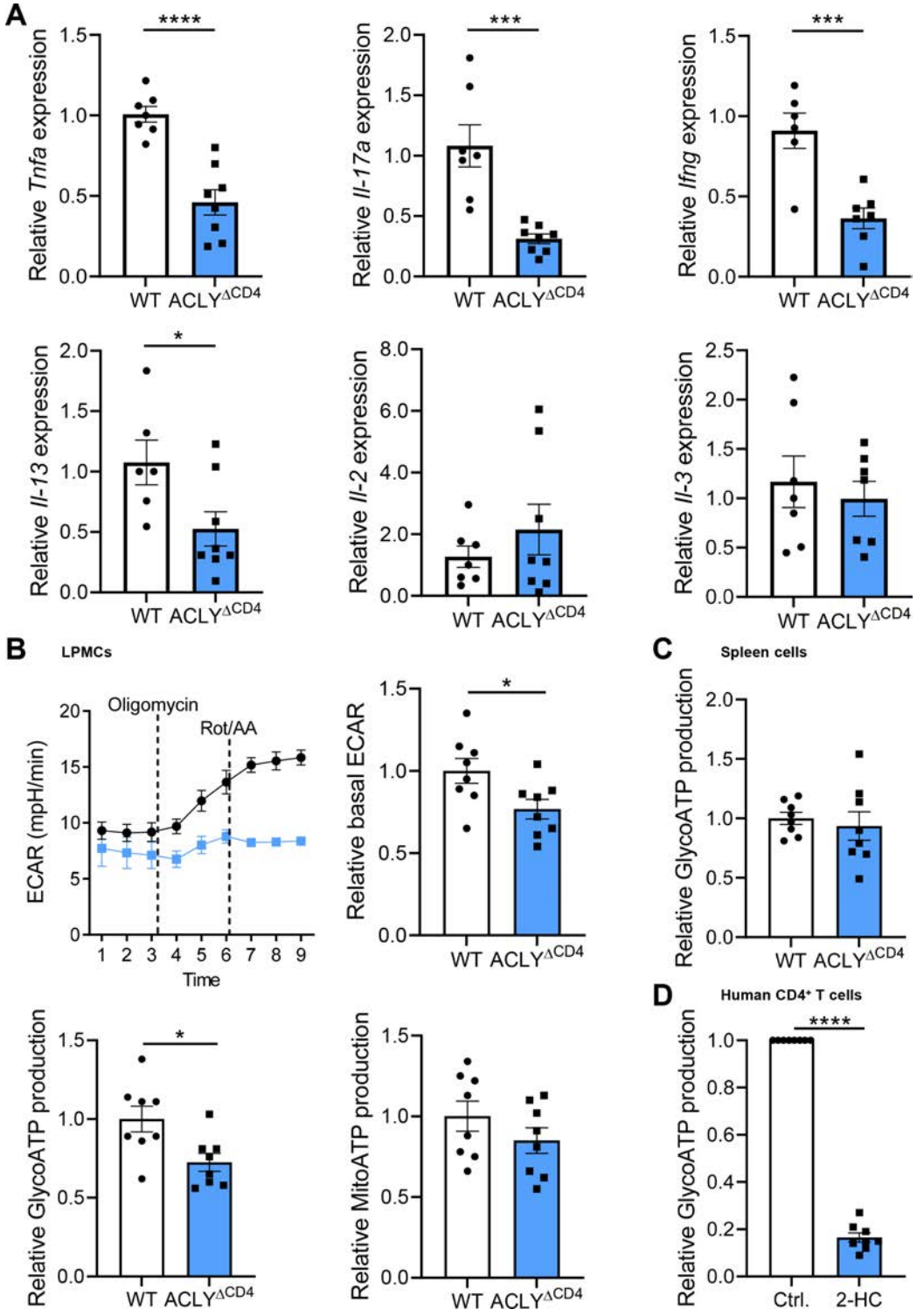

Figure 3 Decreased immunometabolic activity of gut infiltrating ACLY-deficient CD4$^+$ T cells. Experimental colitis was induced by adoptive transfer of ACLY-proficient or ACLY-deficient CD4$^+$CD25$^-$CD44^{low} T cells into *Rag1* KO mice. At the end of the experiment, colonic LPMCs and spleen cells were purified to allow further ex vivo analyses. (A) After 48 hours of in vitro stimulation via anti-CD3/CD28 antibodies, mRNA expression levels of the cytokines *Tnfa*, *Il-17a*, *Ifng*, *Il-13*, *Il-2* and *Il-3* in LPMCs were analysed via qPCR. *Hprt* served as reference gene. Summarising graphs are depicted (single data points and mean±SEM of n=6–8 samples/group; data were normalised based on the mean ΔCT value of the respective control animals). (B) Seahorse technology allowed to monitor the extracellular acidification rate (ECAR) and to determine the GlycoATP and MitoATP production rates of purified LPMCs (Rot/AA for Rotenone/Antimycin A mixture) (C) and the GlycoATP production rate of purified spleen cells. Summarised data (mean±SEM of n=8 samples/group) and a representative ECAR curve are depicted. (D) Blood-derived human CD4$^+$ T cells were stimulated in vitro via anti-CD3/CD28 antibodies in the presence or absence of the ACLY inhibitor 2-HC. Based on Seahorse technology, the GlycoATP production rate was analysed. Summarised data (single data points and mean±SEM of n=8 samples/group) are depicted. Single outliers were identified using the Grubbs test and excluded from further analysis. Unpaired t-test (A–C) or one-sample t-test (D) was applied. *p < 0.05, ***p < 0.001, ****p < 0.0001. LPMCs, lamina propria mononuclear cells.

Gut: first published as 10.1136/gutjnl-2023-330543 on 4 January 2024. Downloaded from http://gut.bmj.com/ on March 18, 2024 at Universitatsbibliothek Erlangen Nurnberg. Protected by copyright.

upregulate cytokine production on stimulation but showed a decreased basal extracellular acidification rate (ECAR) and a significantly reduced glycolysis-dependent ATP (GlycoATP) production already in the absence of additional in vitro stimulation (figure 3B). Interestingly, a similar downregulation of the GlycoATP production rate could not be detected in $CD4^+$ T cell-enriched spleen cells of mice previously transferred with ACLY-deficient T cells (figure 3C). Moreover, ACLY-deficient $CD4^+$ T cells directly purified from the spleens of $ACLY^{\Delta CD4}$ mice showed a normal expression of proinflammatory cytokines on in vitro stimulation (Online supplemental figure 4). It can thus be assumed that the intestinal stimulatory milieu in the model of adoptive transfer colitis can enhance the dependency of immunometabolic T cell activation on ACLY activity.

Interestingly, the requirement of ACLY activity for the sufficient generation of GlycoATP in T cells under stimulatory conditions could also be confirmed for the human immune system. $CD4^+$ T cells purified from the blood of healthy subjects and in vitro stimulated in the presence of the ACLY inhibitor hydroxy citrate (2-HC) showed a significantly reduced GlycoATP production rate (figure 3D). In addition to these alterations in cellular energy production, we also observed a reduced glucose uptake in in vitro stimulated ACLY-deficient murine $CD4^+$ T cells (Online supplemental figure 5A) and in 2-HC-exposed human $CD4^+$ T cells derived from healthy controls or patients with IBD (online supplemental figure 5B). The identified alterations in the glucose metabolism of ACLY-deficient T cells might not only represent a cause of cellular energy shortage but potentially also implicated their reduced capacity to undergo the activation-induced metabolic switch to glycolysis and, thus, to efficiently respond to external stimuli.

Going deeper into the metabolic profiling of ACLY-deficient versus ACLY-proficient LP T cells, untargeted metabolomics of LPMCs purified from the colon of recipient mice transferred with ACLY-deficient T cells showed a marked enrichment of metabolites centrally involved in the biosynthesis of phospholipids and phosphatidylcholine (in particular O-phosphoethanolamine and phosphatidycholine) (figure 4A), and thus also of relevance for the closely related Kennedy pathway, which facilitates de novo synthesis of phosphatidylethanolamine and phosphatidylcholine.[28] The latter metabolic pathway recently turned out to be associated with reduced cellular AcCoA levels in T cells and is functionally connected to the initiation of autophagy.[12] However, our data were not able to detect significantly altered mRNA expression levels of various autophagy-relevant genes in stimulated LPMCs in the absence of ACLY (online supplemental figure 6). Thus, they argued against the assumption that the reduced colitis-inducing capacity of ACLY-deficient $CD4^+$ T cells is driven by an impaired autophagy machinery. Besides the link between the Kennedy pathway and autophagy, there exists good evidence for an anti-inflammatory and colitis-ameliorating effect of its end product phosphatidylcholine. In vivo models implicated its capacity to impact on the colitis-induced remodelling of the intestinal extracellular matrix,[29] and in a phase IIA clinical study, retarded release of phosphatidylcholine was able to increase the histological mucosal healing rate of patients with active ulcerative colitis.[30] In accordance with these data, the observed upregulation of phosphatidylcholine biosynthesis in LPMCs purified from the colon of recipient mice transferred with ACLY-deficient T cells was associated with a significant upregulation of the tryptophan-catabolising enzyme indolamin-2,3-dioxygenase (IDO1) (figure 4B), indicating resolution of inflammation.[31] Moreover, as depicted by label-free multiphoton microscopy, the presence of ACLY-deficient $CD4^+$ T cells seemed to alter the local intestinal collagen structure (figure 4C) and, as indicated by immunofluorescence microscopy, significantly induced local collagen-I expression (figure 4D). This was consistent with regeneration of the extracellular matrix and mucosal healing.[32]

Taken together, the acquired in vivo and ex vivo data are in good accordance with the concept that the inflammation-associated downregulation of ACLY in intestinal T cells represents a counterregulatory immunometabolic process to suppress the colitis-inducing capacity of LP T cells via altering their metabolic profile and flexibility, thereby contributing to the resolution of mucosal inflammation. This implies the targeted inhibition of ACLY in intestinal T cells as a promising strategy for inducing remission in IBD.

Butyrate as a molecular candidate for the therapeutic regulation of ACLY in intestinal T cells

Intending to therapeutically target ACLY in the inflamed gut, we screened defined components of the intestinal microenvironment for their capacity to regulate ACLY expression in in vitro stimulated human T cells. In the applied cell culture setting, the SCFA butyrate interestingly presented a potent negative regulator of ACLY protein expression in human blood-derived $CD4^+$ T cells (figure 5A) and in murine LPMCs (figure 5B), while the presence of the lipid mediator Resolvin E1 or the cytokines IL-3, IL-9 and IL-36α resulted in a marked upregulation of ACLY protein expression (online supplemental figure 7). None of the other tested SCFAs (isobutyrate, acetate and propionate), nor formic acid or other selected IBD-relevant cytkines were able to significantly influence the protein expression level of ACLY in $CD4^+$ T cells (figure 5A, online supplemental figure 7). In accordance with its capacity to downregulate ACLY protein expression in T cells, butyrate induced very similar alterations in the metabolic profile of activated human control- or IBD patient-derived blood $CD4^+$ T cells as before described for ACLY-deficient LP T cells and as observed after inhibition of ACLY in human T cells. Butyric acid-exposed human $CD4^+$ T cells showed significantly decreased basal ECAR levels, reduced ATP production (figure 5C) and an impaired uptake of glucose (figure 5D), while isobutyric acid and formic acid did not induce significant metabolic alterations. In contrast, the presence of butyric acid was unable to further deminish the capacity of ACLY-deficient $CD4^+$ T cells to uptake glucose (figure 5E), thus strongly implicating a dependency of the butyrate-induced metabolic effects in T cells on the capacity of this SCFA to interfere with ACLY activity. In this context, it is also worth mentioning that despite the obvious impact of defined components of the intestinal microenvironment on the expression of ACLY in $CD4^+$ T cells, the colitis-modulating function of the enzyme in intestinal $CD4^+$ T cells was largely maintained also after marked changes of the local micromilieu, as induced by antibiotic therapy and the subsequent reduction of the endogenous gut microbiome (online supplemental figure 8).

Considering the newly identified capacity of butyrate to dampen ACLY expression in purified T cells, we wondered whether this interplay can be transferred and therapeutically applied to the in vivo scenario of chronic colitis. Therefore, taking advantage of the butyrate prodrug tributyrin, whose oral bioavailability and capability to increase intestinal SCFA levels has been documented,[33] we investigated whether the ameliorated intestinal phenotype of *Rag1* KO recipient mice transferred with ACLY-deficient $CD4^+$ T cells in the model of adoptive transfer colitis could be mimicked in fully ACLY-proficient mice

Gut: first published as 10.1136/gutjnl-2023-330543 on 4 January 2024. Downloaded from http://gut.bmj.com/ on March 18, 2024 at Universitatsbibliothek Erlangen Nurnberg. Protected by copyright.

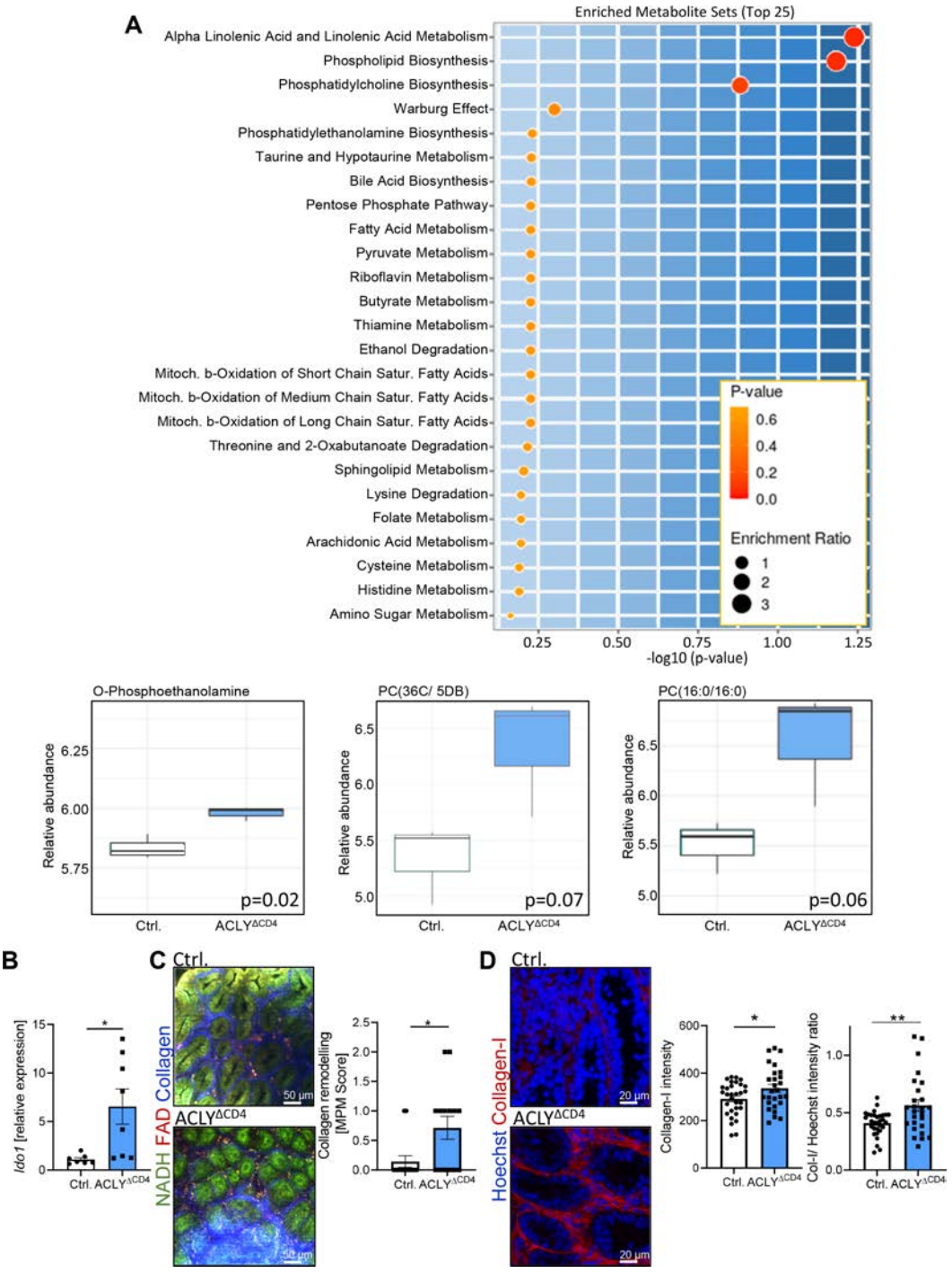

Figure 4 ACLY-deficient intestinal T cells support resolution of inflammation. Experimental colitis was induced by adoptive transfer of ACLY-proficient or ACLY-deficient $CD4^+CD25^-CD44^{low}$ T cells into *Rag1* KO mice. At the end of the experiment, colonic tissue or purified LPMCs were used for further analyses. (A) Based on untargeted metabolomics of n=3 samples per group and supported by the software MetaboAnalyst, the TOP 25 most enriched metabolite sets have been identified in LPMCs purified from the colon of recipient *Rag1* KO mice previously transferred with ACLY-deficient versus ACLY-proficient control cells. The summarising TOP 25 list plus exemplary box-and-whisker plots for 3 selected and relevantly regulated metabolites are shown. (B) After 48 hours of in vitro stimulation via anti-CD3/CD28 antibodies, mRNA expression levels of *Ido1* in LPMCs were analysed via qPCR. *Hprt* served as reference gene. Summarising graph is depicted (single data points and mean±SEM of n=7–8 samples/group; data were normalised based on the mean ΔCT value of the respective control animals). (C) Freshly prepared colon tissue was analysed via label-free multiphoton microscopy. Representative images plus a summarised grading of morphological changes of collagen fibres are shown (n=2 animals per group with seven analysed images per animal; single data points and mean±SEM). (D) The colonic expression pattern of collagen-I (RED) was analysed via immunofluorescence microscopy. Hoechst staining was used to identify cell nuclei (BLUE). Signal intensities for collagen-I and Hoechst were quantified with Fiji software. Representative images and summarising graphs depicting collagen-I signal intensity or the calculated collagen-I/Hoechst intensity ratio are depicted (single data points and mean±SEM; four animals per group; three analysed images per animal; three analysed mucosal areas per image). Single outliers were identified using the Grubbs test and excluded from further analysis. Unpaired t-test was applied. *p < 0.05, **p < 0.01. LMPCs, lamina propria mononuclear cells.

Gut: first published as 10.1136/gutjnl-2023-330543 on 4 January 2024. Downloaded from http://gut.bmj.com/ on March 18, 2024 at Universitatsbibliothek Erlangen Nurnberg. Protected by copyright.

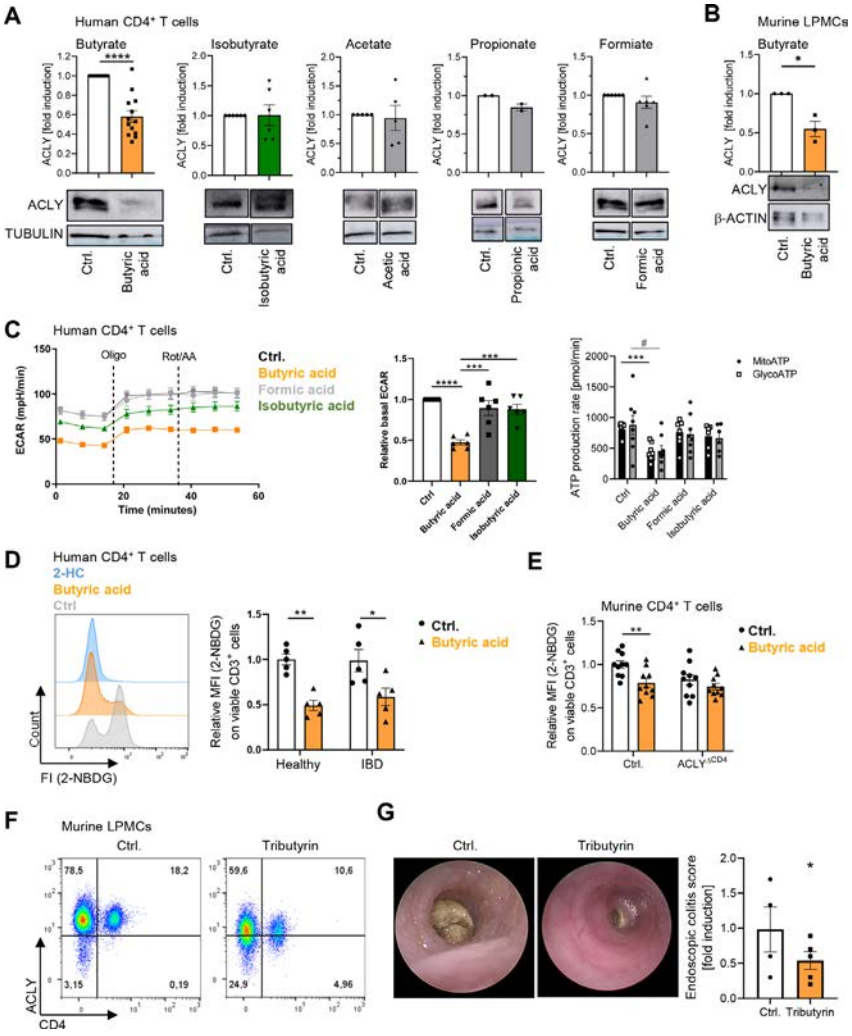

Figure 5 Butyrate as a potent suppressor of ACLY expression in T cells. (A) Human blood CD4$^+$ T cells were stimulated in vitro via anti-CD3/CD28 antibodies in the presence/absence of indicated SCFAs (500 µM) for 72 hours. ACLY expression was analysed via Western Blot. Tubulin served as reference protein and loading control. Graphs summarising data from n=2–13 experiments (single data points and mean±SEM) plus representative images are shown. (B) LPMCs purified from the colon of naïve control mice were stimulated in vitro with anti-CD3/CD28 antibodies in the presence/absence of butyrate for 48 hours. ACLY expression was analysed via Western Blot. β-ACTIN served as reference protein and loading control. A graph summarising data from n=3 mice (single data points and mean±SEM) plus representative images are shown. (C) Human blood-derived CD4$^+$ T cells (healthy donors) were stimulated in vitro via anti-CD3/CD28 antibodies in the presence/absence of indicated SCFA or formic acid for 72 hours. Afterwards, Seahorse technology allowed to monitor the extracellular acidification rate (ECAR) and to determine the GlycoATP and MitoATP production rates. ECAR over time is depicted for one representative donor out of six analysed donors; mean±SEM from three technical replicates for each time point. Graphs summarising data from n=6–8 donors/group (single data points and mean±SEM) are depicted for the relative basal ECAR and the ATP production rate. ***p<0.001, $^{\#}$p<0.05 indicate statistically significant differences for GlycoATP production and MitoATP production, respectively. (D) Human CD4$^+$ T cells derived from the blood of healthy donors or patients with IBD were stimulated in vitro via anti-CD3/CD28 antibodies in the presence/absence of butyrate for 72 hours. The capacity to take up glucose was analysed via flow cytometry. Representative FACS histogram (including 2-HC exposed CD4$^+$ T cells as an additional reference probe) plus a summarising graph (single data points and mean±SEM of n=5 healthy control donors and n=5 patients with IBD) are shown. The two depicted control groups have also been included in the analysis shown in online supplemental figure 5B. (E) Murine CD4$^+$ T cells purified from the spleens of ACLY$^{\Delta CD4}$ mice or respective control animals were stimulated in vitro via anti-CD3/CD28 antibodies in the presence/absence of butyric acid for 72 hours. The capacity to take up glucose was analysed via flow cytometry. Summarised data (single data points and mean±SEM of n=10 mice per group) are shown. (F) Naïve control mice were treated orally with tributyrin (3 mg/kg body weight; twice a day) or PBS (placebo) on three consecutive days. A last tributyrin dose was given on day 4, before mice were euthanised and colonic LPMCs were purified. ACLY expression in LP CD4$^+$ T cells was analysed via flow cytometry. Representative dot plots are depicted. (G) Experimental colitis was induced by adoptive transfer of CD4$^+$CD25$^-$CD44^{low} T cells into *Rag1* KO mice. When mice showed endoscopic signs of colitis (between weeks 3 and 6; inclusion of mice with endoscopic score ≥3), a 10-day period of tributyrin treatment was initiated (3 mg/kg body weight; once a day; oral administration). The control group was treated with PBS. After 10 days, severity of colitis was again scored endoscopically. The fold change of endoscopic colitis score was calculated as quotient of the colitis score before and after treatment. Representative endoscopic pictures and a summarising graph (single data points and mean±SEM of n=4–5 animals per group; analysed in three independent experiments) are shown. Single outliers were identified using the Grubbs test and excluded from further analysis. One-sample t-test (A, B, G) or ANOVA (C–E) was applied. ANOVA, analysis of variance; *p < 0.05, **p < 0.01, ****p < 0.0001; LPMCs, lamina propria mononuclear cells; PBS, phosphate buffered saline; SCFA, short-chain fatty acid.

Gut: first published as 10.1136/gutjnl-2023-330543 on 4 January 2024. Downloaded from http://gut.bmj.com/ on March 18, 2024 at Universitatsbibliothek Erlangen Nurnberg. Protected by copyright.

by therapeutically enhanced intestinal butyrate levels. Indeed, oral tributyrin treatment was able to markedly reduce the frequency of gut-infiltrating ACLY^{high} CD4$^+$ T cells also under in vivo conditions (figure 5F). Moreover, the established colitis in *Rag1* KO mice transferred with ACLY-proficient CD4$^+$ T cells attenuated during a 10-day therapeutic treatment period with tributyrin, as indicated by a significant decrease of the endoscopic colitis score (figure 5G) and also supported by histological scoring (Online supplemental figure 9A). The fact that both the in vivo inhibition of ACLY and the treatment of mice with tributyrin were described to be associated with decreased body weight[34 35] might explain that we did not observe body weight gain in the tributyrin-treated group despite improved colitis (online supplemental figure 9A). In good accordance with the before developed concept that resolution of established chronic colitis is supported by a dampened ACLY activity in colitis-driving T cells, while ACLY function is less relevant for the gut homing of adoptively transferred T cells, prophylactic tributyrin treatment starting before the onset of colitis was not able to prevent intestinal inflammation in the adoptive transfer colitis model (online supplemental figure 9B,C).

Overall, our here described findings were able to newly identify the inflammation-associated downregulation of the metabolic enzyme ACLY in T cells as a colitis-attenuating strategy through which the mucosal immune system can counterregulate chronic colitis and which can be further enhanced therapeutically via the butyrate prodrug tributyrin. Especially patients with IBD with active disease might best benefit from a tributyrin-triggered more pronounced and/or more stable suppression of ACLY protein expression in intestinal CD4$^+$ T cells.

DISCUSSION

Dysregulation of intestinal T cell homoeostasis is a key driver of IBD pathogenesis and a successfully validated therapeutic target.[26 36] However, in order to provide optimised perspectives also to the still too large subgroup of patients with IBD who do not satisfactorily respond to the currently available treatment strategies,[2] the translational development of innovative T cell-targeting therapeutic concepts beyond the blockades of effector cytokines or T cell gut homing remains a critical challenge. In this context, the increasingly considered but not yet fully elucidated impact of immunometabolic signalling cascades on the fate of activated T cells might offer new avenues for controlling the overwhelming T cell response in IBD.[7 37] Following this concept, in this study, we were able to identify a significant involvement of the metabolic enzyme ACLY in the pathogenesis of T cell-driven chronic colitis and described, for the first time, its significant downregulation in CD4$^+$ T cells infiltrating the inflamed gut of patients with IBD.

In general, ACLY is known to be upregulated during T cell activation[13] and subsequently catalyses the generation of extramitochondrial AcCoA from citrate.[11] Besides serving as substrate for de novo lipogenesis and thereby enabling biomass expansion during cell proliferation and influencing the T cell function-modulating process of protein prenylation,[38–40] cytoplasmatic and nuclear AcCoA acts as acetyl group-donor allowing epigenetic gene regulation via histone acetylation but also influencing the function of non-histone proteins of relevance for T cell immune responses.[12 14 15 41 42] Although the specific impact of ACLY on LP T cells in the presence of the complex intestinal microenvironment has not been explicitly analysed before, the in vivo observed reduced colitogenic capacity of gut-infiltrating ACLY-deficient T cells and their impaired production of proinflammatory cytokines was in accordance with published studies describing the dependency of IL2-induced T cell proliferation, optimal Th17 cell differentiation and CD8$^+$ effector cell function on a sufficient availability of cytoplasmatic AcCoA.[11–13 15 42] Somehow unexpected, the reported presence of exogenous acetate in the intestinal micromilieu[43] and its described potency to compensate an inappropriate ACLY-catalysed AcCoA production[22] could not hinder the fact that a significantly reduced severity of experimental colitis was observed after the transfer of ACLY-deficient T cells in the here analysed model of adoptive transfer colitis. This might partly be due to a potential downregulation of local acetate levels on chronic colitis[44] but most likely also reflects that the intestinal expression of the enzyme ACSS2, which catalyses the generation of cytosolic AcCoA from acetate,[45] seems to be mostly restricted to IECs as indicated by the here performed analyses of IBD patient-derived intestinal tissue and by reevaluating the single-cell RNA sequencing data of a publicly available dataset (GSE116222).[23]

Considering the described relevance of ACLY for activated effector T cells[12 13] and our observation that mucosal T cells require ACLY activity for reaching their full proinflammatory capacity, it was surprising, at least at first glance, to realise the inflammation-associated downregulation of ACLY protein expression in T cells infiltrating the inflamed gut tissue of patients with IBD and in experimental colitis, which both represent pathological conditions characterised by increased proinflammatory immune cell activation.[46] However, the in parallel acquired data on the functional behaviour of ACLY-deficient intestinal T cells suggest that the downregulation of the ACLY-mediated metabolic function in T cells on inflammatory conditions represents a counterregulatory mechanism of the mucosal immune system to control and resolve inflammation. Besides the already described decline of ACLY activity during TGF-β-induced T_{reg} differentiation[17] and the here observed decreased colitogenic capacity of ACLY-deficient LP T cells, this conclusion is in particular supported by the finding, that ACLY-deficient LP T cells showed a reduced level of glycolysis-mediated energy production, which is in general associated with anti-inflammatory conditions.[47] Moreover, we were able to describe the induction of *Ido1* mRNA expression in gut-infiltrating T cells in the absence of ACLY as well as a significant modulation of the intestinal collagen structure, representing two phenomena classically contributing to resolution of inflammation.[32 48] Finally, in accordance with the concept that downregulation of ACLY activity in a pathologically altered microenvironment can restrict acute T effector cell responses, increased extracellular potassium levels were found to induce AcCoA shortage in tumour-infiltrating T cells and thereby promote long-term persistency at the expense of exacerbated effector function.[12] Of course, the here developed idea that the inflammation-associated downregulation of ACLY in LP T cells represents an early counterregulatory process to ameliorate intestinal inflammation also raises the question, which compounds of the inflammatory micromilieu can trigger the decrease of ACLY protein levels. While the high complexity of the inflammatory microenvironment in the gut of patients with IBD makes it very challenging to fully answer this question and define the complete panel of involved mediators, our data already implicate that the local tissue levels of the lipid mediator Resolvin E1 might be of relevance in this context. The intracellular synthesis of Resolvin E1 depends on the availability of its precursor, the n-3 polyunsaturated fatty acid eicosapentaenoic acid (EPA).[49] Considering the here observed ACLY-inducing capacity of Resolvin E1 in human CD4$^+$ T cells together with the interesting finding that EPA levels were found

Gut: first published as 10.1136/gutjnl-2023-330543 on 4 January 2024. Downloaded from http://gut.bmj.com/ on March 18, 2024 at Universitatsbibliothek Erlangen Nurnberg. Protected by copyright.

to be significantly downregulated in inflamed gut areas of ulcerative colitis patients,[49] it can be suggested that EPA/Resolvin E1 shortage in inflamed gut tissue relevantly contributes to the inflammation-associated downregulation of ACLY in intestinal T cells of patients with IBD.

With regard to translational perspectives, the identification of the SCFA butyrate as potent suppressor of ACLY expression in activated T cells implies that butyrate-triggered signalling cascades in T cells might represent targets to accelerate and/or maintain downregulation of ACLY in mucosal T cells of patients with IBD and thereby shift overwhelming T cell activation towards resolution of inflammation. Indeed, SCFAs, which are generated during bacterial fermentation of dietary fibres, are already well known as potent modulators of intestinal T cell homoeostasis, influencing the accumulation and function of regulatory T cells and effector T cells.[50 51] In the context of IBD, particular attention has indeed been paid to butyrate. There is a well-documented reduced abundance of butyrate-producing bacterial strains in the microbiome of patients with IBD[52] and several studies demonstrated its favourable effect on T cell homoeostasis and epithelial integrity in colitis.[53 54] Especially in the light of former studies demonstrating an efficient absorption of luminal butyrate through the intact intestinal epithelium[55] and an even increased gut-to-blood penetration of SCFAs in experimental animal models of colitis and in patients with IBD,[56] the orally administrable butyrate-prodrug tributyrin[33] appeared as a promising tool to therapeutically increase local butyrate levels in the LP of inflamed intestinal tissue. By confirming that orally applied tributyrin indeed was not only able to ameliorate chronic colitis in a therapeutic setting, but also markedly reduced the intestinal frequency of ACLY^{high} T cells in in vivo conditions, we could identify an important new aspect underlying the therapeutic potential of butyrate supplementation in IBD, which is the targeted downregulation of ACLY-dependent immunometabolism in intestinal T cells.

In summary, we newly discovered the inflammation-associated downregulation of the metabolic enzyme ACLY in intestinal CD4$^+$ T cells of patients with IBD and, on a functional level, demonstrated that the absence of ACLY in adoptively transferred T cells reduced their colitis-inducing capacity and instead favoured resolution of chronic inflammation. Thus, ACLY appears as a valuable therapeutic target in IBD, whose downregulation in T cells can be achieved by enhancing butyrate signalling and can subsequently control the overwhelming activation of gut-infiltrating T cells by impacting on their immunometabolic behaviour.

Author affiliations
[1]Department of Medicine 1, University Hospital of Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
[2]Current address: Bionorica SE, Neumarkt in der Oberpfalz, Germany
[3]Institute of Experimental and Clinical Pharmacology and Toxicology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
[4]Core Facility for Metabolomics, Department of Medicine, Philipps-Universität Marburg, Marburg, Germany
[5]Division of Molecular Immunology, Department of Internal Medicine 3, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
[6]Institute of Medical Biotechnology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
[7]Department of Medicine 2 – Cardiology and Angiology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
[8]Deutsches Zentrum Immuntherapie DZI, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
[9]Institute for Bioinformatics, Medical University of Innsbruck, Innsbruck, Austria
[10]TRR 241 Research Initiative, Berlin-Erlangen, Germany

Acknowledgements The authors thank for excellent support by the FACS Core Unit of the University Hospital Erlangen.

Collaborators TRR241 IBDome Consortium: Imke Atreya, Raja Atreya, Petra Bacher, Christoph Becker, Christian Bojarski, Nathalie Britzen-Laurent, Caroline Bosch-Voskens, Hyun-Dong Chang, Andreas Diefenbach, Claudia Günther, Ahmed N. Hegazy, Kai Hildner, Christoph S. N. Klose, Kristina Koop, Susanne Krug, Anja A. Kühl, Moritz Leppkes, Rocío López-Posadas, Leif S.-H. Ludwig, Clemens Neufert, Markus Neurath, Jay Patankar, Christina Plattner, Magdalena Prüß, Andreas Radbruch, Chiara Romagnani, Francesca Ronchi, Ashley Sanders, Alexander Scheffold, Jörg-Dieter Schulzke, Michael Schumann, Sebastian Schürmann, Britta Siegmund, Michael Stürzl, Zlatko Trajanoski, Antigoni Triantafyllopoulou, Maximilian Waldner, Carl Weidinger, Stefan Wirtz, Sebastian Zundler.

Contributors AS-K, KR, AJ, MD, PN, SK, JL, L-JL, JB, RVT, LK and BD performed the experiments. AS-K, AG, MFF, DM, MJW, SS, OF, RL-P, CB, SZ, RA, MFN and IA provided clinical samples, protocols, reagents or designed experiments; the TRR241 IBDome consortium provided RNA-sequencing data; AS-K, KR, MD, CP, MFN and IA analysed and interpreted the data; AS-K, MFN and IA drafted the manuscript and the figures; all authors critically revised the manuscript for important intellectual content; IA acts as guarantor.

Funding The here described study was supported by the following grants from the DFG; German Research Foundation: TRR241 (project number 375876048), AT 122/2-1 /project number 511510453) and a DFG sequencing grant to CB (project number 418055832). In addition, this work received funding from the Interdisciplinary Center for Clinical Research Erlangen: IZKF D37.

Competing interests MFN has served as an advisor for Pentax, Giuliani, MSD, Abbvie, Janssen, Takeda and Boehringer. Moreover, MFN serves as an associated editor of the journal Gut. The remaining authors disclose no conflicts.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and the collection of samples was approved by the local ethical committee and the institutional review board of the Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU); reference numbers: 426_20 B; 440_20 B. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

ORCID iDs
Rocío López-Posadas http://orcid.org/0000-0002-2398-5864
Sebastian Zundler http://orcid.org/0000-0003-0888-2784
Christoph Becker http://orcid.org/0000-0002-1388-1041
Raja Atreya http://orcid.org/0000-0002-8556-8433
Markus F Neurath http://orcid.org/0000-0003-4344-1474
Imke Atreya http://orcid.org/0000-0002-2708-9688

REFERENCES

1 Chang JT. Pathophysiology of inflammatory bowel diseases. *N Engl J Med* 2020;383:2652–64.
2 Atreya R, Neurath MF, Siegmund B. n.d. Personalizing treatment in IBD: Hype or reality in 2020? can we predict response to anti-TNF *Front Med*;7.
3 Schreiner P, Neurath MF, Ng SC, *et al*. Mechanism-based treatment strategies for IBD: Cytokines, cell adhesion molecules, JAK inhibitors, gut Flora, and more. *Inflamm Intest Dis* 2019;4:79–96.
4 Jain N, Walker WA. Diet and host-microbial Crosstalk in postnatal intestinal immune homeostasis. *Nat Rev Gastroenterol Hepatol* 2015;12:14–25.
5 Adolph TE, Zhang J. Diet fuelling inflammatory bowel diseases: Preclinical and clinical concepts. *Gut* 2022;71:2574–86.
6 Laudisi F, Stolfi C, Monteleone G. Impact of food additives on gut homeostasis. *Nutrients* 2019;11:2334.
7 Zaiatz Bittencourt V, Jones F, Doherty G, *et al*. Targeting immune cell metabolism in the treatment of inflammatory bowel disease. *Inflamm Bowel Dis* 2021;27:1684–93.
8 Pålsson-McDermott EM, O'Neill LAJ. Targeting Immunometabolism as an anti-inflammatory strategy. *Cell Res* 2020;30:300–14.

Gut: first published as 10.1136/gutjnl-2023-330543 on 4 January 2024. Downloaded from http://gut.bmj.com/ on March 18, 2024 at Universitatsbibliothek Erlangen Nurnberg. Protected by copyright.

9 Lee M, Chang EB. Inflammatory bowel diseases (IBD) and the Microbiome-searching the crime scene for clues. *Gastroenterology* 2021;160:S0016-5085(20)35507-4:524–37.:.
10 Singer BD, Chandel NS. Immunometabolism of pro-repair cells. *J Clin Invest* 2019;129:124613:2597–607.:.
11 Dominguez M, Brüne B, Namgaladze D. Exploring the role of ATP-citrate Lyase in the immune system. *Front Immunol* 2021;12:632526.
12 Vodnala SK, Eil R, Kishton RJ, *et al*. T cell Stemness and dysfunction in tumors are triggered by a common mechanism. *Science* 2019;363:eaau0135.
13 Hochrein SM, Wu H, Eckstein M, *et al*. The glucose transporter Glut3 controls T helper 17 cell responses through Glycolytic-epigenetic Reprogramming. *Cell Metab* 2022;34:S1550-4131(22)00087-0:516–532..
14 Balmer ML, Ma EH, Bantug GR, *et al*. Memory Cd8(+) T cells require increased concentrations of acetate induced by stress for optimal function. *Immunity* 2016;44:S1074-7613(16)30105-4:1312–24.:.
15 Osinalde N, Mitxelena J, Sánchez-Quiles V, *et al*. Nuclear Phosphoproteomic screen Uncovers ACLY as mediator of IL-2-induced proliferation of Cd4+ T lymphocytes. *Mol Cell Proteomics* 2016;15:2076–92.
16 Berod L, Friedrich C, Nandan A, *et al*. De Novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. *Nat Med* 2014;20:1327–33.
17 Tian M, Hao F, Jin X, *et al*. ACLY Ubiquitination by Cul3-Klhl25 induces the Reprogramming of fatty acid metabolism to facilitate iTreg differentiation. *Elife* 2021;10:e62394.
18 Zorzi F, Monteleone I, Sarra M, *et al*. Distinct profiles of Effector Cytokines mark the different phases of Crohn's disease. *PLoS One* 2013;8:e54562.
19 Fantini MC, Becker C, Tubbe I, *et al*. Transforming growth factor beta induced Foxp3+ regulatory T cells suppress Th1 mediated experimental colitis. *Gut* 2006;55:671–80.
20 Wartewig T, Daniels J, Schulz M, *et al*. PD-1 instructs a tumor-suppressive metabolic program that restricts Glycolysis and restrains AP-1 activity in T cell lymphoma. *Nat Cancer* 2023;4:1508–25.
21 Riley SA, Mani V, Goodman MJ, *et al*. Comparison of delayed-release 5-Aminosalicylic acid (Mesalazine) and sulfasalazine as maintenance treatment for patients with ulcerative colitis. *Gastroenterology* 1988;94:1383–9.
22 Zhao S, Torres A, Henry RA, *et al*. ATP-citrate Lyase controls a glucose-to-acetate metabolic switch. *Cell Rep* 2016;17:S2211-1247(16)31325-0:1037–52.:.
23 Parikh K, Antanaviciute A, Fawkner-Corbett D, *et al*. Colonic epithelial cell diversity in health and inflammatory bowel disease. *Nature* 2019;567:49–55.
24 Izzo LT, Trefely S, Demetriadou C, *et al*. Acetylcarnitine shuttling links mitochondrial metabolism to Histone Acetylation and Lipogenesis. *Sci Adv* 2023;9:eadf0115eadf0115.
25 Kuna RS, Kumar A, Wessendorf-Rodriguez KA, *et al*. Inter-Organelle cross-talk supports acetyl-coenzyme A homeostasis and Lipogenesis under metabolic stress. *Sci Adv* 2023;9:eadf0138eadf0138.
26 Neurath MF. Current and emerging therapeutic targets for IBD. *Nat Rev Gastroenterol Hepatol* 2017;14:269–78.
27 Wirtz S, Popp V, Kindermann M, *et al*. Chemically induced Mouse models of acute and chronic intestinal inflammation. *Nat Protoc* 2017;12:1295–309.
28 Gibellini F, Smith TK. The Kennedy pathway--de novo synthesis of Phosphatidylethanolamine and Phosphatidylcholine. *IUBMB Life* 2010;62:414–28.
29 Mourelle M, Guarner F, Malagelada JR. Polyunsaturated Phosphatidylcholine prevents Stricture formation in a rat model of colitis. *Gastroenterology* 1996;110:1093–7.
30 Karner M, Kocjan A, Stein J, *et al*. "First multicenter study of modified release Phosphatidylcholine "LT-02" in ulcerative colitis: a randomized, placebo-controlled trial in Mesalazine-refractory courses". *Am J Gastroenterol* 2014;109:1041–51.
31 Onali S, Favale A, Fantini MC. The resolution of intestinal inflammation: the peace-keeper's perspective. *Cells* 2019;8:344.
32 Ramadass SK, Jabaris SL, Perumal RK, *et al*. Type I collagen and its daughter peptides for targeting Mucosal healing in ulcerative colitis: A new treatment strategy. *European Journal of Pharmaceutical Sciences* 2016;91:216–24.
33 Edelman MJ, Bauer K, Khanwani S, *et al*. Clinical and pharmacologic study of Tributyrin: an oral butyrate prodrug. *Cancer Chemother Pharmacol* 2003;51:439–44.
34 Samsoondar JP, Burke AC, Sutherland BG, *et al*. Prevention of diet-induced metabolic dysregulation, inflammation, and Atherosclerosis in Ldlr(-/-) mice by treatment with the ATP-citrate Lyase inhibitor Bempedoic acid. *Arterioscler Thromb Vasc Biol* 2017;37:647–56.
35 Sato FT, Yap YA, Crisma AR, *et al*. Tributyrin attenuates metabolic and inflammatory changes associated with obesity through a Gpr109A-dependent mechanism. *Cells* 2020;9:2007.
36 Hammoudi N, Hamoudi S, Bonnereau J, *et al*. Autologous Organoid Co-culture model reveals T cell-driven epithelial cell death in Crohn's disease. *Front Immunol* 2022;13:1008456.
37 Michaudel C, Danne C, Agus A, *et al*. Rewiring the altered Tryptophan metabolism as a novel therapeutic strategy in inflammatory bowel diseases. *Gut* 2023;72:1296–307.
38 Chypre M, Zaidi N, Smans K. ATP-citrate Lyase: a mini-review. *Biochem Biophys Res Commun* 2012;422:1–4.
39 Zaidi N, Swinnen JV, Smans K. ATP-citrate Lyase: a key player in cancer metabolism. *Cancer Res* 2012;72:3709–14.
40 López-Posadas R, Fastancz P, Martínez-Sánchez LDC, *et al*. Inhibiting Pggt1B disrupts function of RHOA, resulting in T-cell expression of integrin Alpha4Beta7 and development of colitis in mice. *Gastroenterology* 2019;157:1293–309.
41 Puccini J, Wei J, Tong L, *et al*. Cytoskeletal Association of ATP citrate Lyase controls the Mechanodynamics of Macropinocytosis. *Proc Natl Acad Sci U S A* 2023;120:e2213272120.
42 Chowdhury S, Kar A, Bhowmik D, *et al*. Intracellular acetyl Coa potentiates the therapeutic efficacy of antitumor Cd8+ T cells. *Cancer Res* 2022;82:2640–55.
43 Macfarlane S, Macfarlane GT. Regulation of short-chain fatty acid production. *Proc Nutr Soc* 2003;62:67–72.
44 Treem WR, Ahsan N, Shoup M, *et al*. Fecal short-chain fatty acids in children with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 1994;18:159–64.
45 Vysochan A, Sengupta A, Weljie AM, *et al*. Acss2-mediated acetyl-Coa synthesis from acetate is necessary for human cytomegalovirus infection. *Proc Natl Acad Sci U S A* 2017;114:E1528–35.
46 Neurath MF. Cytokines in inflammatory bowel disease. *Nat Rev Immunol* 2014;14:329–42.
47 Michalek RD, Gerriets VA, Jacobs SR, *et al*. Cutting edge: distinct Glycolytic and lipid oxidative metabolic programs are essential for Effector and regulatory Cd4+ T cell Subsets. *J Immunol* 2011;186:3299–303.
48 Royzman D, Andreev D, Stich L, *et al*. Soluble Cd83 triggers resolution of arthritis and sustained inflammation control in IDO dependent manner. *Front Immunol* 2019;10:633.
49 Pearl DS, Masoodi M, Eiden M, *et al*. Altered Colonic Mucosal availability of N-3 and N-6 polyunsaturated fatty acids in ulcerative colitis and the relationship to disease activity. *Journal of Crohn's and Colitis* 2014;8:70–9.
50 Smith PM, Howitt MR, Panikov N, *et al*. The microbial metabolites, short-chain fatty acids, regulate Colonic Treg cell homeostasis. *Science* 2013;341:569–73.
51 Fischer A, Zundler S, Atreya R, *et al*. Differential effects of Alpha4Beta7 and Gpr15 on homing of Effector and regulatory T cells from patients with UC to the inflamed gut in vivo. *Gut* 2016;65:1642–64.
52 Takahashi K, Nishida A, Fujimoto T, *et al*. Reduced abundance of butyrate-producing bacteria species in the fecal microbial community in Crohn's disease. *Digestion* 2016;93:59–65.
53 Silva JPB, Navegantes-Lima KC, Oliveira ALB, *et al*. Protective mechanisms of butyrate on inflammatory bowel disease. *Curr Pharm Des* 2018;24:4154–66.
54 Segain JP, Raingeard de la Blétière D, Bourreille A, *et al*. Butyrate inhibits inflammatory responses through Nfkappab inhibition: implications for Crohn's disease. *Gut* 2000;47:397–403.
55 Dinges MM, Lytle C, Larive CK. 1)H NMR-based identification of Intestinally absorbed metabolites by Ussing chamber analysis of the rat Cecum. *Anal Chem* 2018;90:4196–202.
56 Jaworska K, Konop M, Bielinska K, *et al*. Inflammatory bowel disease is associated with increased gut-to-blood penetration of short-chain fatty acids: A new, non-invasive marker of a functional intestinal lesion. *Exp Physiol* 2019;104:1226–36.

Gut: first published as 10.1136/gutjnl-2023-330543 on 4 January 2024. Downloaded from http://gut.bmj.com/ on March 18, 2024 at Universitatsbibliothek Erlangen Nurnberg. Protected by copyright.