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Alterations in intestinal fatty acid metabolism in inflammatory bowel disease

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Abstract

Inflammatory bowel disease (IBD) constitutes a severe intestinal disorder in developed countries with increasing incidence worldwide. Upcoming evidence indicates an important role of intestinal epithelial barrier function in the development of IBD. Fatty acids exert nutritional and protective effects on enterocytes, serve as activators of transcription and constitute precursors of inflammatory mediators. The aim of this study was to investigate differential regulation of genes involved in fatty acid uptake and endogenous fatty acid biosynthesis in IBD. Mucosal biopsy specimens from non-affected regions of the intestine were subjected to DNA microarray analysis. Gene array analysis revealed a variety of genes involved in fatty acid uptake and synthesis to be differentially expressed in ileum and colon of selected IBD patients. To verify these results, real-time RT-PCR was performed for selected regulated candidate genes in larger IBD sample numbers. In single biopsy analysis long chain acyl-CoA synthetase (ACSL) 1 and 4 were upregulated in IBD ($P<0.05$), while a significant decrease in fatty acid synthase expression was found in ileum and colon of ulcerative colitis patients ($P<0.001$). Expression of the transcription factor liver X receptor (LXR) which was previously shown to induce fatty acid synthase gene expression was not altered on mRNA level in IBD. However, in cell culture experiments using the human intestinal cell line LS174T induction of fatty acid synthase by the LXR ligand T0901317 was inhibited by TNFα. Moreover, these experiments indicated a decrease of LXR protein levels by TNFα treatment. These data suggest that the decrease of fatty acid synthase expression in ulcerative colitis patients could be at least partially due to a loss of LXR expression and function in the presence of pro-inflammatory cytokines. Observed alterations in expression of genes of fatty acid metabolism may contribute to the pathophysiology of ulcerative colitis.

Keywords: Fatty acid transport; Fatty acid synthase; Inflammatory bowel disease; Cytokine; Liver X receptor

1. Introduction

Many aspects of the multifactorial etiology and pathogenesis of inflammatory bowel diseases (IBD), ulcerative colitis (UC) and Crohn's disease (CD), still remain poorly understood. In addition to some identified genetic factors, such as the NOD2 gene [1,2], it is assumed that exaggerated immune responses to the normal gut microflora involving CD4+ T-cells are mainly responsible for initiation and perpetuation of chronic intestinal inflammation [1]. In particular, secretion of proinflammatory molecules such as TNFα obviously play a central role in the pathogenesis of IBD, since the blockade of TNF with specific antibodies has been shown to be an effective treatment in CD [2,3]. However, there is upcoming evidence that the intestinal epithelial cell itself plays an important role in triggering local immune responses, as well as in providing a barrier function between the intestinal lumen and immune cells [4]. Therefore, the integrity and function of the intestinal epithelium seems to constitute an important factor in the development of IBD. A wide range of different fatty acids are essential factors for enterocyte physiology and inflammatory processes [5,6].

Short-chain fatty acids (SCFA), in particular butyrate, which is produced in the gut by microbial fermentation of carbohydrates and endogenous substrates [7], such as mucus, serve as important energy source for colonic mucosal cells. Beneficial effects of SCFA on inflamed colonic mucosa including UC have been demonstrated [8], while a lack of butyrate can result in intestinal inflammation [9]. The mechanisms underlying the protective role of SCFA are not fully understood so far. Butyrate

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metabolism is involved in a variety of biochemical processes including detoxification of xenobiotics [10] and mucus synthesis [11]. In addition, butyrate has been described to reduce colonic paracellular permeability by enhancing peroxisome proliferator-activated receptor γ (PPARγ) activation [12].

Besides SCFA, long chain fatty acids considerably contribute to enterocyte function and pathology. In particular, polyunsaturated fatty acids (PUFA), such as ω-3 and ω-6 PUFA, are able to modulate inflammatory processes. In IBD, beneficial effects of ω-3 fatty acids like eicosapentaenoic acid and docosahexaenoic acid have been shown in several clinical trials [13]. ω-3 fatty acids were demonstrated to affect tight junction permeability of intestinal epithelial cells [14] and to decrease oxidative stress in UC patients [15]. By competing with ω-6 fatty acids, eicosapentaenoic acid inhibits the production of arachidonic acid metabolites like leukotriene B4 and prostaglandin E2 via the 5-lipoxygenase pathway [16]. However, prostaglandin E2 and its receptor EP4, attenuate dextran sodium sulphate-induced (DSS) colitis in animal models by reducing the levels of mucosal inflammatory cytokines and suppressing T-cell activation in the gut [17–19]. Recently, conjugated linoleic acid (CLA), a class of positional, geometric, conjugated dienoic isomers of linoleic acid was also demonstrated to ameliorate DSS colitis in a mouse model by activation of PPARγ resulting in repression of tumor necrosis factor α (TNFα) expression and NFκB activation, while the immunoregulatory cytokine transforming growth factor β (TGFβ) was induced [20]. In UC patients, PPARγ displayed a diminished expression in colonic epithelial cells [21] and reversal of reduced PPARγ expression in mice with DSS-induced colitis markedly attenuated disease activity [22].

Although increasing evidence indicates an important role of fatty acids and PPARγ in health and pathology of the intestinal mucosa, alterations of fatty acid metabolism itself are only poorly understood so far. Therefore, we here addressed the question, how intestinal fatty acid uptake as well as fatty acid synthesis and metabolism are altered in the intestinal epithelium of IBD patients. Therefore, the expression of fatty acid transport proteins (FATP), fatty acid binding proteins (FABP), long chain acyl-CoA synthetases (ACSL) as well as genes involved in endogenous fatty acid synthesis were analyzed in intestinal epithelial cells of IBD patients.

2. Material and methods

2.1. Patients and tissue specimens

Approval conduct to the study was obtained from the Ethics Committee of the University of Regensburg. Samples for real-time RT-PCR were derived from biopsies taken from terminal ileum and colon from 13 patients with Crohn's disease, 19 patients with ulcerative colitis and 16 control patients, who underwent coloscopy for other reasons than IBD, including tumor staging (Table 1). In 11 patients of the control group coloscopy revealed no tissue abnormalities, two patients had diverticulosis, one patient diverticulitis and two patients suffered from infectious colitis. All diagnoses of IBD patients were based on classical clinical features, radiological, endoscopic and laboratory findings. Disease activity was based on endoscopic criteria. A special diet was not administered. Patients were fasting for at least 12 h before coloscopy. IBD tissue biopsies from non-inflamed regions 10 cm distant from pathological area were selected and sampled by a specialized gastroenterologist. Biopsies were immediately stabilized in RNAlater solution (Qiagen, Hilden, Germany) and kept at −80 °C until isolation of RNA.

Table 1
Patient information

Disease activity	Non-IBD	Crohn's disease		Ulcerative colitis	
		Active	Inactive	Active	Inactive
n	16	8	5	16	3
Sex (M/F)	8/8	3/5	2/3	11/5	2/1
Age (years)					
Range	22–69	23–59	36–74	20–72	33–38
mean	47.2	41.7	57.2	39.1	35.3

2.2. RNA isolation

Total RNA was extracted from the tissue biopsies or cultured cell lines according to the manufacturer's instructions using the RNeasy Protect midi Kit (Qiagen, Hilden, Germany). Purity and integrity of the RNA was assessed on the Agilent bioanalyzer with the 6000 Nano LabChip reagent set (Agilent Technologies, USA). The RNA was quantified spectrophotometrically and then stored at −80 °C.

2.3. Cell culture

The human adenocarcinoma cell line LS174T was obtained from ATCC (Manassas, USA). Cells were grown in Dulbecco's Modified Eagle medium supplemented with 10% heat-inactivated fetal calf serum, 2 mmol/l glutamine and 1% (wt/vol) penicillin/streptomycin (Gibco BRL, Gaithersburg, MD, USA). Cultures were maintained at 37 °C in a humidified 5% CO_2 atmosphere. Cells were stimulated for 24 h with 100 ng/ml human recombinant TNFα expressed in E. coli (Sigma, St. Louis, USA), and/or 10 μM T0901317 (Sigma, St. Louis, USA) or 10 μM rifampicin (Sigma, St. Louis, USA).

2.4. DNA microarray analysis

DNA microarray analysis was performed as described earlier [23]. Gene expression profiles were determined using Affymetrix HGU133A and HGU133B GeneChips covering 22238 annotated human genes and more than 33000 human EST sequences. Arrays were performed using a pool from total RNA of four specimens from each patient and control group. Validation of mucosal control genes indicated that the gene expression profiles of the analyzed biopsy specimens mainly represent the global gene expression of epithelial cells and that immune cells do not significantly contribute to the observed transcript patterns [23]. Microarray data were deposited in the GEO database.

2.5. Real-time RT-PCR (TaqMan™) analysis

First-strand cDNA synthesis was performed with the Reverse Transcription System from Promega (Madison, USA) according to the manufacturer's instructions. Real-time RT-PCR analysis was performed with an ABI7900HT machine (Applied Biosystems). All TaqMan RT-PCR reagents, including primers and probes, were purchased from Applied Biosystems. TaqMan analysis was performed with predesigned and optimized Assays on Demand (Applied Biosystems). The following assays were used: CD36 (ID: Hs00169627_m1), FATP1-6 (IDs: Hs00417419_g1, Hs00186324_m1, Hs00225680_m1, Hs00192700_m1, Hs00202073_m1, Hs00204034_m1), FABP1-7 (IDs: Hs00155026_m1, Hs00164552_m1, Hs00269758_m1, Hs00609791_m1, Hs00870436_s1, Hs00155029_m1, Hs00361426_m1), FABP-PM (ID: Hs00751057_s1), ACSL1 (ID: Hs00242530_m1), ACSL3-6 (IDs: Hs00244853_m1, Hs00244871_m1, Hs00212106_m1, Hs00362960_m1), fatty acid synthase (ID: Hs00188012_m1), IL-8 (ID: Hs00174103_m1), 18S ribosomal RNA (ID: Hs99999901_s1), ABCA1 (ID: Hs00194045_m1),

CYP3A4 (ID: Hs00430021_m1), steaoryl-CoA desaturase (ID: Hs00748952_s1), SREBP1 (ID: Hs00231674_m1), LXRα and β (IDs: Hs00172885_m1, Hs00173195_m1), TNF (Hs00174128_m1), NFκB1 (Hs00231653_m1), RelA (Hs00153294_m1), TGFβ1 (Hs99999918_m1). The reaction parameters were as follows: 2-min 50 °C hold, 30-min 60 °C hold, and 5-min 95 °C hold, followed by 45 cycles of 20-s 94 °C melting and 1-min 60 °C annealing/extension. Measurements were performed in duplicates or triplicates. Results were analyzed with an ABI sequence detector software version 2.0 (Applied Biosystems). Relative quantitation was performed as described earlier [24], and 18S ribosomal RNA (rRNA) was used as a reference gene. Since all assays used were optimized for PCR efficiency by the manufacturer, mRNA expression levels were estimated by delta ct values.

2.6. Immunoblotting

The following antibodies were used: rabbit anti-human fatty acid synthase (Assay Designs, Michigan, USA), rabbit anti-human LXRα/β (H-144) (Santa Cruz Biotechnology, Santa Cruz, USA), mouse anti-human villin (Chemicon International, Hofheim, Germany). Cells were harvested, washed with PBS and frozen (−20 °C). Cells were lysed by 0.02% SDS solution and sonicated (MSE Soniprep 150, Beun de Ronde BV, Abcoude, The Netherlands). Equal amounts of protein were analyzed by SDS-PAGE using 4–12% gels. After electrophoresis, proteins were transferred to fluorotrans transfer membranes (Pall, Dreieich, Germany). Membranes were then blocked by incubation for 1 h in PBS containing 5% nonfat milk powder and 0.1% Tween 20. For detection of individual proteins, membranes were incubated for 1 h with the corresponding antibodies in a dilution of 1:1000 in PBS with 1% nonfat milk powder and 0.1% Tween 20. Detection of immune complexes was carried out with the ECL plus Western blot detection system (Amersham Pharmacia, Deisenhofen, Germany) and analyzed with a Lumi-Imager (Roche Diagnostics, Mannheim, Germany).

2.7. Statistical analysis

All data are expressed as mean±S.D. Statistical analysis was performed using SPSS 12.0 software. Statistical significance was determined by the Mann–Whitney *U* test for biopsy data and the Student *t* test for paired samples for cell culture experiments. The level of significance was corrected to Bonferroni to compensate for the effect of multiple comparisons. A $P<0.05$ was considered as statistically significant.

3. Results

3.1. mRNA expression of genes involved in fatty acid uptake and activation in normal human terminal ileum and colon

Fatty acid uptake, cellular binding and activation to acyl-CoA for subsequent metabolic conversion obviously play a central role for enterocyte physiology. To evaluate the normal mRNA expression pattern of these genes real-time RT-PCR was performed for the FATP, FABP and ACSL family members using RNA isolated from biopsies of 5–10 control specimens taken from colon and terminal ileum. To compare the mRNA expression of different genes in the two tissues, expression levels are displayed as filled circles in Table 2, which are based on delta ct values (ct (target gene) -ct (18S rRNA). A small interindividual variability in gene expression was observed with the exception of FABP6. Within the FATP family, FATP2 and FATP4 displayed highest expression levels in terminal ileum and in colon, whereas FATP5 and FATP6 were only weakly or not expressed in both tissues. As expected from literature data, FABP1 and FABP6 exhibited highest expression levels in the ileum. However, considerable expression levels in this tissue were also detected for FABP2, FABP5 and FABP-PM. In the colon, FABP1 mRNA was abundantly present, whereas FABP6 was clearly weaker expressed. In addition, we found significant mRNA expression signals for FABP2, FABP5 and FABP-PM. Considerable mRNA amounts for ACSL1, ACSL3, ACSL4 and ACSL 5 were detected in both tissues. Weaker expression levels were detected for ACSL6. These data clearly show a substantial expression of several genes needed for effective fatty acid uptake and synthesis mechanisms in intestine. In particular, FATP4, FABP1, FABP2 and ACSL1, 3 and 4 were highly expressed.

Table 2
mRNA expression of FATPs, CD36, FABPs and ACSLs in terminal ileum and colon of control specimens

	Terminal ileum	Colon
FATP1	●●●	●●●
FATP2	●●●●	●●●
FATP3	●●	●●
FATP4	●●●●	●●●
FATP5	●	not expressed
FATP6	●	●
FAT/CD36	●●●	●●●
FABP-PM	●●●	●●●
FABP1	●●●●●	●●●●●
FABP2	●●●●	●●●●
FABP3	●	●●
FABP4	●●	●●
FABP5	●●●	●●●
FABP6	●●●●	●●
FABP7	not expressed	●
ACSL1	●●●	●●●●
ACSL3	●●●●	●●●●
ACSL4	●●●	●●●
ACSL5	●●●●	●●●●
ACSL6	●●	●

mRNA expression of FATPs, CD36, FABPs and ACSLs in terminal ileum and colon of control specimens. Expression levels are represented as filled circles and are based on delta ct levels. At least five different tissue specimens were analyzed for each gene.

3.2. Genes involved in fatty acid metabolism are differentially regulated in IBD

We next addressed the question whether these genes involved in fatty acid metabolism were differentially regulated in IBD. In order to obtain the global mRNA expression profiles of all these genes, pooled RNAs of biopsies from terminal ileum and colon taken from four CD and UC patients and controls, respectively, were subjected to DNA microarray analysis and further evaluated using a biomedical pathway approach. Probesets detecting FATP2–6 and CD36, FABP1–7, ACSL1, ACSL3–6 as well as genes and regulators of fatty acid synthesis were present on the array. Estimating basal gene expression levels from array probeset data, highest expression levels were found for FABP1 and FABP6 which was consistent with real-time RT-PCR results. Several genes, including FATP5, FATP6, FABP3, FABP4, and FABP7 could not be detected by the array (Table 2).

Array results indicated an increase of ACSL1 and ACSL4 expression (fold change: up to 4.0 and up to 2.5) in colon of CD and UC patients. In contrast, fatty acid synthase was decreased in the colon of IBD patients (up to −5.7 fold). Moreover, the transcriptional master regulator of fatty acid synthesis, sterol regulatory element-binding protein 1 (SREBP1), was down-regulated in the colon of CD and UC patients (−2.1 fold and −2.8 fold, respectively). The differential regulations were also observed in the ileum and colon of CD patients, but to a lower extent. No change could be observed for the mRNA expression of the transcription factors liver X receptor (LXR) α and β, which were previously shown to modulate the expression of fatty acid synthase as well as of SREBP1 [25–27] (Table 3). To confirm these findings from DNA microarrays, we performed quantitative real-time RT-PCR analysis in a larger number of single unpooled and unrelated biopsy specimens (4–14 independent samples for each patient group) for selected genes. Single biopsy analysis revealed a significant increase of ACSL1 and ACSL4 expression in the ileum and colon of both, CD and UC patients ($P<0.05$) (Fig. 1A, B). In addition, real-time RT-PCR revealed a down-regulation of fatty acid synthase gene expression in terminal ileum and colon of UC patients ($P<0.001$) (Fig. 1C). A tendency showing a decrease of SREBP1 expression in the colon of UC patients could be observed (Fig. 1D).

To evaluate if an impaired expression of LXR isoforms was responsible for the observed loss of fatty acid synthase and SREBP1 gene expression in UC we investigated the expression

Table 3
Expression and regulation of genes involved in fatty acid metabolism and TNFα signaling in CD or UC samples compared with control specimens

Gene	RefSeq	Control expression of ileum	FC CD	FC UC	Control expression of colon	FC CD	FC UC
Fatty acid transport and binding							
FATP2	NM_003645	188	1.5	1.1	185	1.1	1.0
FATP3	BC003654	88	− 1.3	1.3	108	− 1.6	− 1.4
FATP4	AK000722	415	1.6	− 1.3	413	− 2.3	− 4.9
FATP5	AL042852	n.e.			n.e..		
FATP6	NM_014031	n.e.			n.e.		
FAT/CD36	AW299226	153	2.8	1.3	127	2.3	1.1
FABP-PM	NM_002080	423	− 1.1	− 1.4	453	1.0	1.0
FABP1	NM_001443	5545	− 1.6	− 1.5	4867	1.1	1.0
FABP2	NM_000134	46	1.5	− 1.1	76	− 1.9	− 4.9
FABP3	AI219891	n.e.			n.e.		
FABP4	NM_001442	n.e.			n.e.		
FABP5	NM_001444	596	− 1.3	− 2.2	1068	1.1	1.1
FABP6	U19869	5666	− 1.3	− 1.1	3545	− 1.1	− 10.6
FABP7	NM_001446	n.e.			n.e.		
Fatty acid acetylation							
ACSL1	NM_021122	33	1.1	1.2	88	1.7	4.0
ACSL3	AL525798	159	1.5	− 1.1	190	1.7	1.3
ACSL4	NM_022977	11	1.6	1.1	25	1.7	2.5
ACSL5	AW173691	3499	− 1.1	− 1.1	2438	− 1.2	− 1.3
ACSL6	AL390168	25	1.2	− 1.2	23	1.2	1.1
Fatty acid synthesis							
Fatty acid synthase	AI954041	110	− 2.0	− 1.2	177	− 2.6	− 5.7
LCE	NM_024090	112	1.2	1.6	332	2.1	1.6
SCD	AB032261	166	1.1	− 1.1	111	1.4	2.1
SREBP1	NM_004176	127	1.1	− 1.1	128	− 2.1	− 2.8
SCAP	NM_012235	185	− 1.1	− 1.3	213	− 1.3	− 1.3
LXRα	NM_005693	128	1.2	1.3	128	− 1.5	1.1
LXRβ	NM_007121	53	− 1.2	1.3	n.e.		
TNFα signaling							
TNF	NM_000594	n.e.			n.e.		
TNF receptor-1A	NM_001065	596	1.3	1.0	573	− 1.1	− 1.2
TNF receptor-1B	NM_001066	81	− 1.1	1.3	63	− 1.3	− 2.0
FADD	NM_003824	74	1.1	1.1	114	− 1.1	− 1.2
TRADD	L41690	57	− 1.3	1.5	72	− 1.1	1.4
IKKβ	AU153366	106	1.1	1.9	137	1.0	1.3
IKKγ	NM_003639	213	− 1.4	1.0	182	− 1.4	− 1.6
IKAP	NM_001556	97	1.3	1.2	95	1.5	1.4
NFKB1	M55643	208	− 1.5	1.0	186	− 1.9	1.0
TGF-β1	BC000125	62	− 1.6	1.4	73	− 1.5	− 3.7

Expression and regulation of genes involved in fatty acid uptake, activation, and in fatty acid synthesis in CD or UC samples compared with control specimens. The control expression values are given for the terminal ileum and the colon. Genes with average difference intensity of 50 are considered as significantly expressed in the given cell type. The fold change (FC) of gene expression between controls and patient groups is indicated. n.e.: not expressed.

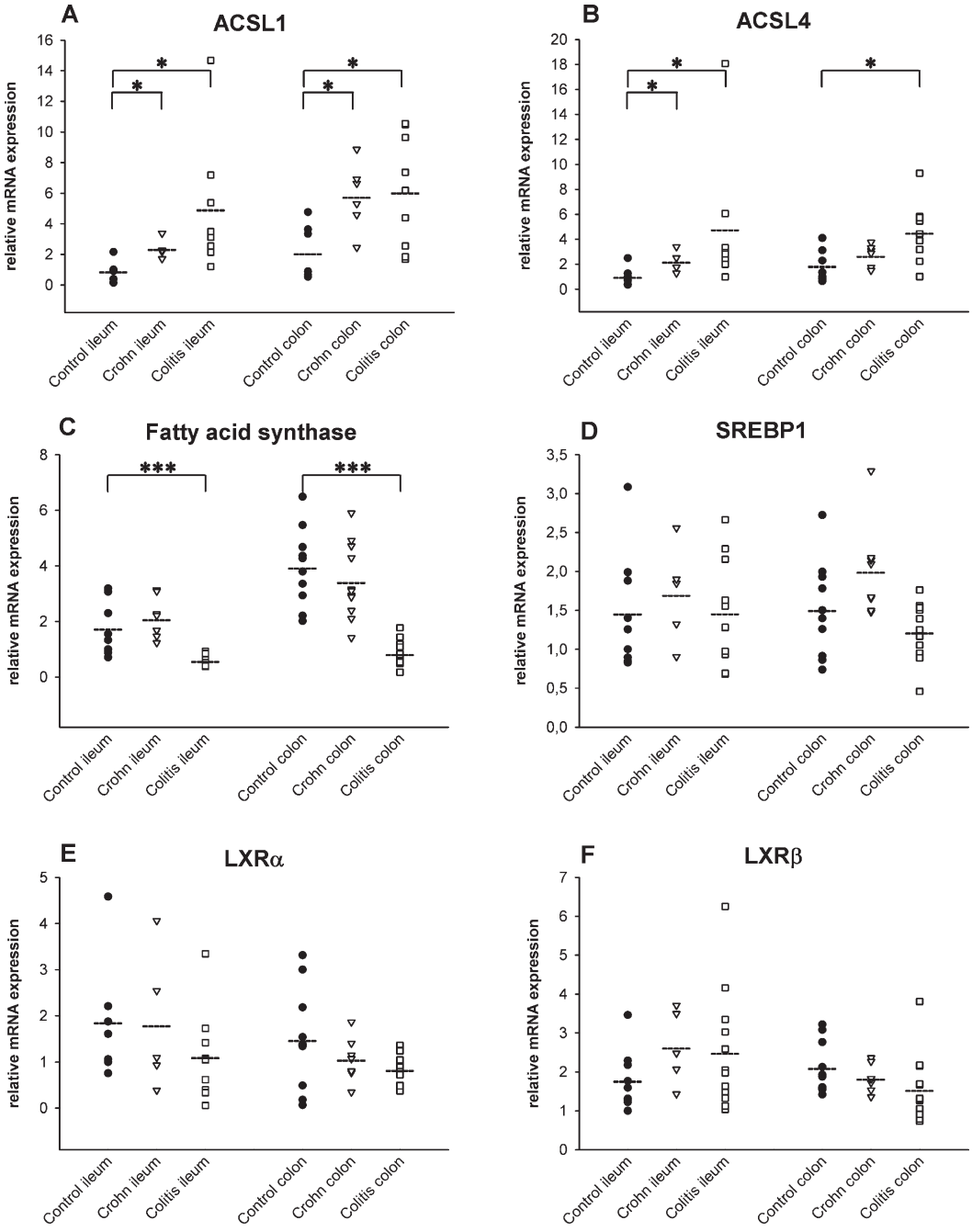

Fig. 1. mRNA expression of ACSL1 (A), ACSL4 (B), fatty acid synthase (C), SREBP1(D) and LXRα and β (E and F) in IBD biopsy specimens. Real-time RT-PCR analysis both genes in ileal and colonic biopsy samples from control individuals (black circle) and CD (open triangle) and UC patients (open square). Transcript abundance of analyzed genes was expressed as relative gene expression. Expression levels were normalized to 18S rRNA. Measurements were performed in duplicate. The horizontal line indicates the mean gene expression value of each group. *$P<0.05$ (CD or UC versus control), ***$P<0.001$ (UC versus control).

of both LXR isoforms, LXRα and LXRβ, in single biopsies. However, in accordance with gene array data, expression of LXRα and LXRβ was not significantly altered in IBD (Fig. 1E, F).

The decrease of FATP4, CD36 and FABP6 gene expression indicated by array analysis of pooled samples could not be verified by real-time RT-PCR of single biopsies (data not shown). Discrepancies may be due to interindividual scatter of mRNA expression levels as well as to detection of certain mRNA splice variants by the gene array.

3.3. Effect of TNFα on LXR target gene expression in LS174T cells

To elucidate underlying mechanisms of the strongly decreased expression of fatty acid synthase in colitis patients we investigated whether the down-regulation of LXR targets like fatty acid synthase and SREBP1 could be potentially mediated by pro-inflammatory cytokines, such as TNFα, or if ligand-induced LXR activation could be altered by TNFα. We incubated LS174T cells with human recombinant TNFα and/

or the PXR ligand rifampicin or the synthetic LXR ligand T0901317 for 24 h. After stimulation, mRNA levels of several genes as well as fatty acid synthase protein levels were analyzed. As positive controls, we selected IL-8, known to be upregulated at the mRNA level in intestinal cell lines [28], and CYP3A4 that was demonstrated to be induced by PXR ligands [23]. As indicated in Fig. 2A, stimulation with TNFα caused a strong increase in IL-8 mRNA expression. CYP3A4 was induced by rifampicin stimulation and this effect was strongly inhibited by addition of TNFα. We could also observe a strong upregulation

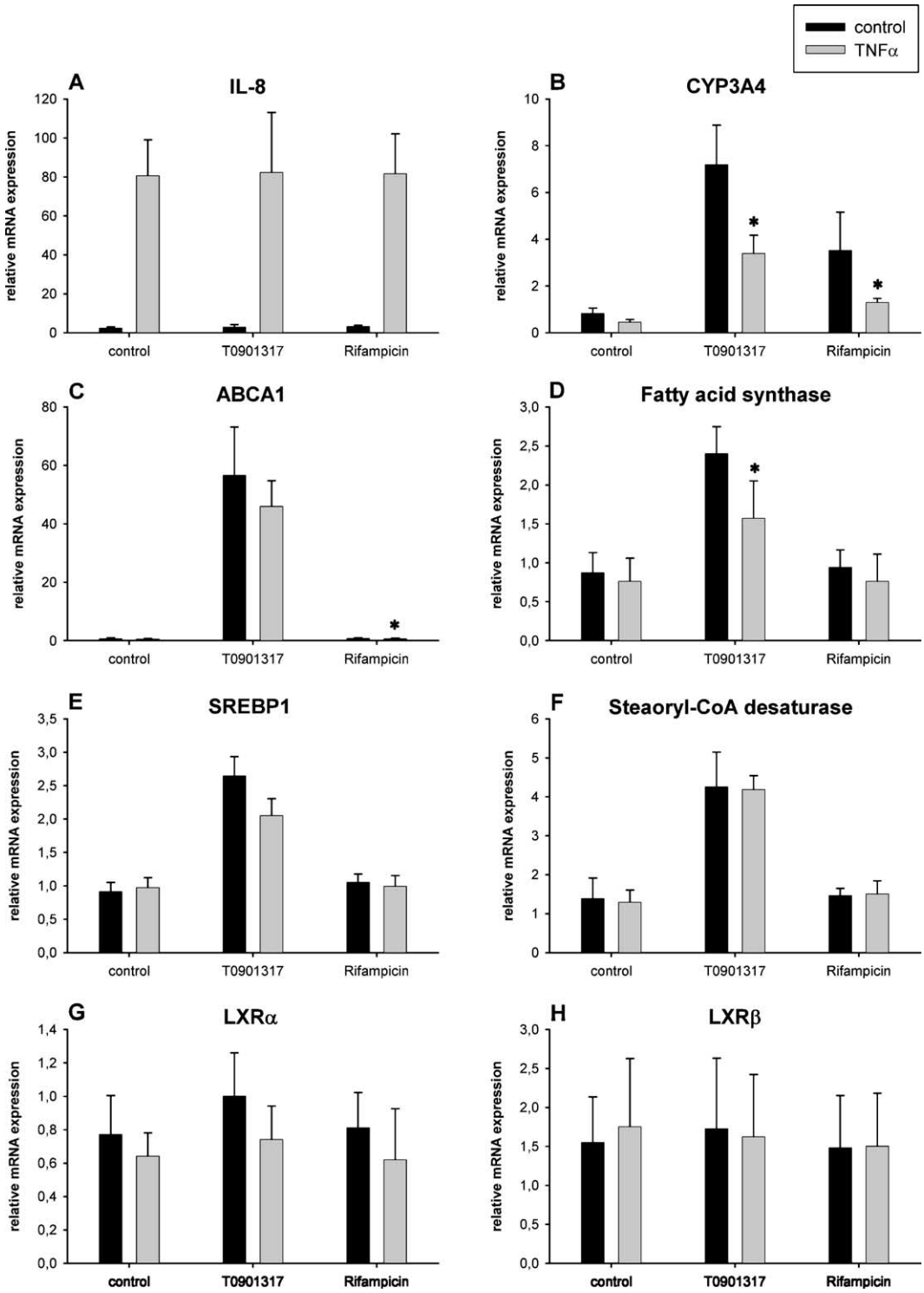

Fig. 2. Inhibition of LXR ligand-induced gene expression by TNFα. LS174T cells stimulated with human recombinant 100 ng/ml TNFα and/or 10 μM rifampicin or 10 μM T0901317 for 24 h and gene expression of IL-8 (A), CYP3A4 (B), ABCA1 (C) fatty acid synthase (D), SREBP1(E) and steoryl-CoA desaturase (F), LXRα (G) and LXRβ (H) was monitored by real-time RT-PCR. The results of RT-PCR analysis were expressed as relative gene expression and normalized to 18S rRNA expression as reference gene. Results are shown as means±S.D. of 3 individual experiments. Measurements were performed in triplicate. *$P<0.05$ (TNFα versus control).

of CYP3A4 using the LXR ligand T0901317 instead of rifampicin which is most likely due to PXR activation by T0901317 [29]. Again, induction was inhibited by addition of TNFα (Fig. 2B).

As expected, stimulation with T0901317 induced gene expression of the analyzed LXR target genes ABCA1, fatty acid synthase, steaoryl-CoA desaturase and SREBP1 after 24 h, while rifampicin did not alter the expression of these genes. Costimulation with T0901317 and TNFα clearly impaired the LXR ligand-induced mRNA expression of ABCA1 and fatty acid synthase (Fig. 2C, D). Inhibition of T0901317-induced fatty acid gene expression could also be observed at the protein level by immunoblotting (Fig. 3A). In tendency, the same regulation was found for SREBP1, but not for steaoryl-CoA desaturase (Fig. 2E, F). However, basal expression of these genes was not influenced by TNFα.

In the same cell culture model, we investigated the expression of LXRα and β. Similar to the gene expression patterns in patient biopsies, LXRα and β mRNA expression was not affected by stimulation with TNFα. However, TNFα treatment reduced LXR protein levels in total cell lysate (Fig. 3B). These date indicate that in this cell type inflammatory cytokines may mainly alter LXR translation and probably LXR function, but not LXR mRNA expression.

3.4. Genes involved in TNFα signaling are not altered on the mRNA level

Our cell culture experiments indicated a role of TNFα in differential regulation of fatty acid synthase expression in UC patients. Therefore, we additionally evaluated the mRNA expression of genes of TNFα signal transduction in these patients. TNF mRNA could not be detected by gene array experiments and was found only weakly expressed by real-time RT-PCR in analyzed biopsies (Table 3 and data not shown). Moreover, microarray analysis did not indicate differential regulation of genes involved in intestinal TNFα signal transduction [30] ,including TNF receptor-1A and -1B, Fas-associated death domain (FADD), TNF receptor-1-associated death domain (TRADD), IκB kinase (IKK)-β and -γ, IKK complex-associated protein (IKAP) and NFκB1 (Table 3). Array data for NFκB1 as well as RelA which was not detected by the array were validated in single biopsies (data not shown).

TGF-β1 expression was found decreased in colon of CD and UC patients (−1.5 fold and −3.7 fold, respectively) by gene array experiments. However, this result could not be verified by real-time RT-PCR in single biopsies (data not shown).

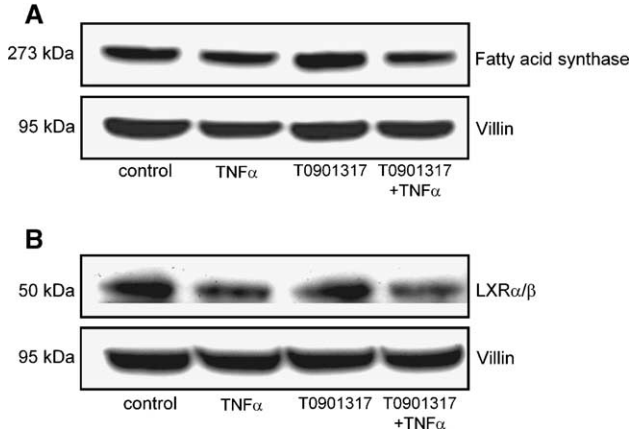

Fig. 3. Inhibition of LXR expression and LXR ligand-induced fatty acid synthase expression by TNFα. LS174T cells stimulated with human recombinant 100 ng/ml TNFα and/or 10 μM T0901317 for 24 h and protein expression of fatty acid synthase (A) and LXRα/β (B) was detected by SDS page. Protein expression of villin was used as reference gene. Results of one representative experiment are shown.

4. Discussion

Fatty acids play an important role in inflammatory processes as well as in ensuring nutrition and cellular integrity by providing a molecular source for phospholipid synthesis. Active uptake of long chain fatty acids involves FATPs (solute carrier family 27 1–6). This family of integral transmembrane proteins consists of six so far identified members which show different tissue expression patterns [31]. Among these genes, FATP4 has been identified as the major intestinal transport protein that is expressed at high levels on the apical side of mature enterocytes in the small intestine and is required for efficient uptake of fatty acids by enterocytes, mainly palmitate and oleate [32]. Our studies revealed in addition to FATP4 high expression levels of FATP2, suggesting a significant role of this transporter in intestinal tissues as well. Moreover, CD36 is well characterized in facilitating fatty acid uptake in a variety of tissues [33]. Although, data from CD36-deficient mice suggest only a minor role of this transporter in enterocytes [34], considerable mRNA amounts of CD36 were detected in the analyzed ileum and colon biopsies from human samples. Moreover, recent evidence by Drover et. al. indicates that CD36 is important for secretion and clearance of intestinal lipoproteins [35]. Therefore, a species difference cannot be excluded.

Further mechanisms of intestinal absorption of long chain fatty acids involve the family of FABPs which reversely bind fatty acids after entering the cell for further fatty acid metabolism. Besides the supply of fatty acids, FABPs may also protect the cell against the detergent effects of free fatty acid and bile acids. Seven different FABPs have been described so far, however, major roles in the intestinal tissue have been attributed only to FABP1 (L-FABP) that is preferentially implicated in phospholipid synthesis, membrane protection and gene regulation, FABP2 and FABP6 (gastropin, ileal) [36,37]. This is in good accordance with our data detecting high levels of FABP1, FABP2 and FABP6 in terminal ileum biopsies and high mRNA amounts of FABP1 and FABP2 in colon biopsies.

For further fatty acid metabolism acetylation of fatty acids by ACSL is required. Five human ACSL isoforms have been identified so far. Substrates of this enzyme family can enter a variety of pathways including synthesis of fatty acids, triacylglycerol and phospholipids, β-oxidation and cholesterol

esterification. Interestingly, ACSL1 and 4 seem to provide acyl-CoA destinated primarily for triacylglycerol and de-novo synthesis of phospholipids, while β-oxidation is suggested to rely on acyl-CoA synthesized by other isoforms [38]. In our study, we found a significant increase of ACSL1 and ACSL4 in terminal ileum and in colon of IBD patients. This implies that enterocyte fatty acids may mainly enter the phospholipid pathway in CD and UC patients, where they could serve as precursors for inflammatory mediators. Moreover, phospholipids may be incorporated into intestinal plasma membranes supporting membrane integrity and epithelial barrier function of the affected intestine. Therefore, upregulation of ACSL1 and ACSL4 could be a compensatory mechanism for slowing disease progression in IBD.

Besides fatty acid uptake, de-novo synthesis of long-chain saturated and monounsaturated fatty acids significantly contributes to the production of membrane phospholipids [39]. In colorectal neoplasia both, the transcription factor SREBP1 and fatty acid synthase are highly upregulated [40], most likely to provide membrane phospholipids for these rapidly proliferating cells [41,42]. SREBP1 plays a central role in regulating the biosynthesis of proinflammatory mediators, as well as in controlling the endogenous synthesis of saturated and monounsaturated fatty acids, involving fatty acid synthase, long chain fatty acyl elongase and stearoyl-CoA desaturase [43]. Fatty acid synthase is the key enzyme of endogenous fatty acid synthesis leading to palmitate (C16:0), stearic acid (C18:0) and oleic acid (C18:1). Interestingly, Ehehalt et al. could demonstrate that the most abundant phosphatidylcholine (PC) species in rectal mucus were PC 16:0/18:1, PC 16:0/18:2, PC 18:0/18:1 and PC 18:0/18:2 [44], of which almost all are potential products of fatty acid synthase activity. In our analyzed biopsies we found a highly significant decrease of fatty acid synthase expression in ileum and colon of UC patients. Therefore, the finding that patients with inactive UC showed significant less PC in rectal mucus [44] explaining increased susceptibility of enterocytes, may be a direct consequence of impaired fatty acid synthase expression in these patients. Since the hydrophobic mucus layer plays a key role in intestinal barrier properties [45], down-regulation of fatty acid synthase may significantly contribute to the pathophysiology of UC in this context.

However, the mechanisms leading to decreased fatty acid synthase expression still remain unclear. In addition to SREBP1, LXR significantly contributes to the transcriptional regulation of endogenous fatty acid synthesis. In particular, SREBP1 [26,27], fatty acid synthase and steaoryl-CoA desaturase are also induced by LXR [25,46]. LXR belongs to the family of nuclear hormone receptors which function as ligand-activated transcription factors [47,48]. Transcriptional activity of these receptors requires heterodimerization with the retinoid X receptor (RXR) [49]. The two LXR isoforms, LXRα and LXRβ, are well known to act as key regulators in sterol and fatty acid metabolism, transactivating a variety of genes that govern transport, catabolism and elimination of cholesterol. Different oxysterols represent naturally occurring ligands for these receptors [50]. In addition to its central role in lipid metabolism, evidence also suggests an involvement of LXR in inflammatory processes. In rodent liver, LPS treatment induces a decrease of LXR expression, as well as of its heterodimerization partner RXR [51] and significantly impairs mRNA levels of the LXR targets ATP-binding cassette (ABC) transporter ABCG5 and ABCG8 [52–54]. Both genes were also found to be down-regulated in colon of UC patients [23]. In J774 murine macrophages endotoxins or cytokines also resulted in a decrease of ABCA1 and ABCG1 [52], two further LXR target genes [55,56]. Moreover, in irritant and contact models of dermatitis, LXR activators display potent anti-inflammatory activity requiring the participation of LXRα and LXRβ [57].

In addition to LXR, further members of the nuclear hormone receptor family have been implicated to the regulation of inflammatory processes, in particular IBD, including PPARγ and pregnane X receptor (PXR).

A recent study from our group could demonstrate a loss of expression of the transcription factor PXR in epithelial cells of colon of UC patients compared with control specimens. This down-regulation was accompanied by a decreased expression of a set of PXR target genes, including ABCB1 (MDR1), mainly involving cellular detoxification and defense [23].

Because of the growing evidence that nuclear hormone receptors play, an important role in inflammatory processes, we here addressed the question, if LXR-induced fatty acid synthase activation was impaired by inflammatory mediators, such as TNFα. Although TNFα signaling showed no transcriptional changes in IBD, an induction of this pathway by other mechanisms cannot be excluded. The finding that TNF mRNA was only weakly expressed and not differentially regulated in analyzed biopsies emphasizes the role of recruited immune cells as source of proinflammatory cytokines in IBD [30].

Using cell culture model for intestinal epithelial cells we found a significant reduction of LXR mediated fatty acid synthase expression upon stimulation with TNFα. mRNA expression of LXRα and LXR β remained unaffected under these conditions while we observed decreased protein expression potentially involved down-regulation of fatty acid synthase. However, further studies are required to elucidate underlying mechanisms in this context as well as functional aspects, e.g. alterations in nuclear translocation. Nevertheless, our results do not fully explain the observed strongly down-regulated fatty acid synthase mRNA expression in biopsies of UC patients. These findings suggest that the loss of fatty acid synthase expression in UC may in part be due to a loss of LXR protein expression and also LXR function in an inflamed environment, but seems to be additionally caused by unknown mechanisms.

In summary, we here identified considerable alterations in the expression of genes involved in intestinal fatty acid metabolism in IBD patients. The observed changes in the expression pattern of genes involved in fatty acid uptake, metabolism and synthesis may be partially a consequence of inflammation, but also seem to contribute substantially to the pathophysiology of the disease. However, further investigation including measurement of enzyme activities and lipid analysis will be needed to elucidate causative pathomechanisms.

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