Inflammatory Bowel Disease Alters Intestinal Bile Acid Transporter Expression

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ABSTRACT

The enterohepatic circulation of bile acids (BAs) critically depends on absorption of BA in the terminal ileum and colon, which can be affected by inflammatory bowel disease (IBD). Diarrhea in IBD is believed to result in part from BA malabsorption (BAM). We explored whether IBD alters mRNA expression of key intestinal BA transporters, BA detoxifying systems, and nuclear receptors that regulate BA transport and detoxification. Using real-time polymerase chain reaction, mucosal biopsy specimens from the terminal ileum in Crohn’s disease (CD) patients and from the descending colon in ulcerative colitis (UC) patients were assessed for mRNA expression. Levels were compared with healthy controls. The main ileal BA uptake transporter, the apical sodium dependent bile acid transporter, was downregulated in active CD and UC and in CD in remission. Other significant changes such as repression of breast cancer–related protein and sulphotransferase 2A1 were seen only during active disease. In UC, pancolitis (but not exclusively left-sided colitis) was associated with altered expression of major BA transporters [multidrug resistance–associated protein 3 (MRP3), MRP4, multidrug resistance gene 1, organic solute transporter a/b] and nuclear receptors (pregnane X receptor, vitamin D receptor) in the descending colon. UC pancolitis leads to broad changes and ileitis to selective changes in intestinal BA transporter expression. Early medical manipulation of intestinal BA transporters may help prevent BAM.

Introduction

Under physiologic conditions, bile acids (BAs) cycle several times a day between the intestine and liver. Due to efficient conservation within the enterohepatic circulation, only small amounts of BA escape reabsorption in the ileum and colon and must be replaced by hepatic de novo synthesis (Hofmann, 1999; Kullak-Ublick et al., 2004). BA uptake into the enterocyte occurs principally in the terminal ileum via the apical sodium dependent BA transporter (ASBT; SLC10A1 gene). Secondary BA produced by bacteria in the colon enter the colonic enterocyte exclusively by passive diffusion (Hofmann, 1999). Several exporters proteins at enterocyte basolateral and apical membranes pump both primary and secondary BA into the blood or the gut lumen (see Fig. 1 for details) in both the terminal ileum and colon. Every enterocyte contains detoxification systems [e.g., cytochrome p450 enzymes such as CYP3A4 or sulphating enzymes such as sulphotransferase 2A1 (SULT2A1)] that metabolize not only xenobiotics but also BA, protecting enterocytes from accumulation of BA in potentially harmful concentrations (Langmann et al., 2004).

Both BA transporters and elements of the enterocytic detoxification system are regulated by feedback mechanisms (Trauner and Boyer, 2003). Farnesoid X receptor (FXR), a nuclear receptor for BA, plays a key role in the functional interaction of transporters (Makishima et al., 1999; Parks et al., 1999). Activation of FXR in enterocytes by BA leads, inter alia, to downregulation of BA uptake pumps and upregulation of BA efflux pumps. Fibroblast growth factor 19 (FGF-19) mediates the feedback regulation between the gut and liver. After stimulation by BA, FGF-19 is produced in the ileal enterocyte, secreted into the portal circulation, and taken up by hepatocytes, where it downregulates BA synthesis (Inagaki et al., 2005). Reduced expression of one BA transporter is often associated with compensatory enhanced or reduced expression of others, which limits BA toxicity (Claudel et al., 2011).

Various diseases, genetic disorders, or drugs may induce or inhibit expression of transporters and metabolizing enzymes, leading to disturbed feedback mechanisms (Roberts et al., 2002). Mutations in both copies of ASBT lead to primary (congenital) BA-induced malabsorption (BAM) with diarrhea, steatorrhea, and hypocholesterolemia (Oelkers et al., 1997). Patients with diarrhea-predominant irritable bowel syndrome (IBS) seem to suffer from BAM due to...
impaired FGF-19 feedback inhibition, resulting in excessive BA synthesis with overloaded capacity for ileal reabsorption (Walters et al., 2009). Secondary BAM results from ileal resection [e.g., due to Crohn’s disease (CD)] with consequently reduced ileal BA reabsorption and presumable missing FGF-19–mediated suppression of hepatic BA synthesis. Inflammatory bowel diseases [IBDs; viz., CD and ulcerative colitis (UC)] are chronic relapsing disorders characterized by an aberrant inflammatory response with mucosal inflammation and tissue damage. IBD is associated with diarrhea, malnutrition, and weight loss through inhibited absorption of nutrients, electrolytes, and water (Sands, 2007). BAM in ileal CD was also observed in surgically untreated CD (Mehoff and Kern, 1968; Tougaard et al., 1986; Lenicek et al., 2011). However, exact molecular mechanisms for BAM in IBD are incompletely understood (Nolan et al., 2013).

We designed this study to test the hypotheses that 1) ileitis due to CD and 2) colitis due to UC disturb intestinal BA homeostasis by deregulation of ileal/colonic BA transport and detoxifying systems, and that 3) changes in expression levels of mRNA in species involved in these systems and their regulation may persist even during remission of IBD.

![Schematic overview of the enterohepatic circulation of BAs with details for intestinal BA transporters, enzymes, and key regulatory nuclear receptors.](image)

**Materials and Methods**

**Study Design**

We conducted a prospective, single-center study comparing findings in enteric mucosal biopsy specimens from IBD patients with active disease, from such patients in remission, and from control patients without demonstrable disease. The study was approved by the Medical University Graz ethics committee (application 18-082 ex 06/07).

**Patient Characteristics**

**Adults.** A total of 137 consecutive patients undergoing endoscopic procedures in the Endoscopy Unit of the Division of Gastroenterology and Hepatology, Medical University Graz, were invited to participate in our study over a period of 27 months. Among these 137 patients, 21 with CD, 14 with UC, and 9 healthy control subjects were enrolled after giving informed consent (Supplemental Fig. 1). Before sampling colonoscopy, clinical data from cases and controls were collected from clinical, endoscopy, and histopathology reports. Clinical data included age, gender, indication for colonoscopy, type of IBD, age at diagnosis of IBD, extent and severity of disease, concomitant diseases, history of ileal or colonic surgery, use of medication (current and past medication ever used), and surveillance details. Control patients underwent endoscopy for diverse indications (Table 1); some reported irregular stooling patterns or periodic abdominal pain, others required screening for colorectal
cancer. Included in the study were only sex- and age-matched control subjects with macroscopically and histologically normal mucosa and without evidence of an underlying gastrointestinal disorder or any other disease. The principal indications for colonoscopy in IBD patients were increasing symptoms (worsening abdominal or joint pain; more frequent diarrhea, nausea, or vomiting). The study excluded pregnant and lactating women and persons with inconclusive histologic findings or other severe disease (e.g., cancer). Details of concomitant diseases and intestinal surgeries antedating colonoscopies are listed in Table 1. Most IBD patients were receiving typical therapy [systemic or topical corticosteroids, aminosalicylates, immunosuppression, or biologics (Table 2)]. Other medications were calcium-cholecalciferol as prophylaxis against osteoporosis or lornoxicam for joint pain.

Adolescents. Collection of additional biopsy specimens was necessary to complement our initial data with data on ileal FGF-19 and FXR expression levels. For technical reasons, expression analyses in stored adult samples were no longer possible. Due to possible confounding factors in adults (e.g., medications, history of surgery), we decided to extend the collection of biopsy specimens to therapy-naive adolescents (age 13–18 years). Adolescents with newly diagnosed CD ileitis (n = 3) or UC pancolitis (n = 7) were compared with healthy children (n = 6) undergoing colonoscopy due to abdominal pain. In these treatment-naive patients, we assessed ileal FGF-19, FXR, and ASBT expression levels in both active ileal CD and UC pancolitis without ileal inflammation.

Laboratory Analysis. To obtain indirect evidence of disease activity via a laboratory index, we determined serum concentrations of the routinely assessed inflammation marker C-reactive protein (CRP), which correlates well with IBD activity (Vermeire et al., 2006). CRP values in healthy controls were within expected ranges (<9 mg/l) and were elevated in patients with active IBD but not in patients with IBD in remission (Table 3). Leukocyte counts, inconsistently elevated in IBD, served as nonspecific markers for other inflammatory conditions and stressful events. Among all groups with active IBD in adults, only patients with UC and pancolitis had leukocyte counts significantly higher (P = 0.03) than those in healthy controls (Table 3). Laboratory analyses in adolescents are not shown.

TABLE 1

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Control</th>
<th>CD Ileitis</th>
<th>Remission</th>
<th>UC Pancolitis</th>
<th>Distal UC</th>
<th>Remission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution of patients</td>
<td>Number of patients</td>
<td>9</td>
<td>14</td>
<td>7</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>5/4</td>
<td>4/10</td>
<td>7/—</td>
<td>2/3</td>
<td>2/2</td>
<td>1/4</td>
</tr>
<tr>
<td>Age (mean in years)</td>
<td>38 ± 9</td>
<td>30 ± 10</td>
<td>39 ± 6</td>
<td>37 ± 19</td>
<td>43 ± 20</td>
<td>50 ± 16</td>
</tr>
</tbody>
</table>

Indication for colonoscopy
- Surveillance: 4
- Abdominal pain: 2
- Stooling irregularities\(a\): 3
- Flare of IBD: —

Symptoms before colonoscopy
- Free of symptoms: 4
- Diarrhea (stool frequency >3/day): 2
- Abdominal pain: 5
- Nausea/vomiting: —
- Painful arthropathy: —

Surgical history
- Ileal resection: —
- Colorectal resection: —
- Anal fistula: —
- Perianal abscess: —
- Colorectal polypectomy: —

\(a\)Diarrhea with stool frequency >3/day and/or obvious blood in the feces.

TABLE 2

<table>
<thead>
<tr>
<th>List of concomitant medications</th>
<th>Control (n = 9)</th>
<th>CD Ileitis (n = 14)</th>
<th>Remission (n = 7)</th>
<th>UC Pancolitis (n = 5)</th>
<th>Distal UC (n = 4)</th>
<th>Remission (n = 5)</th>
</tr>
</thead>
</table>

Drugs taken before sampling colonoscopy
- Prednisolone: —
- Budesonide: —
- Mesalazine: —
- Sulfasalazine: —
- Azathioprine: —
- 6-Thioguanin: —
- Infliximab: —
- Adalimumab: —
- Etanercept: —
- Calcium-cholecalciferol: —
- Lornoxicam: —

Drugs discontinued for more than 6 months
- Prednisolone: —
- Azathioprine: —
- Methotrexate: —
- Infliximab: —
- Sulfasalazine: —
Sample Collection. During endoscopy, extent of disease was categorized by type and extent of inflammation: CD with active ileitis (n = 14) with or without colitis, CD in remission of ileitis (n = 7), distal UC (limited to descending colon and analogous to left-sided UC; n = 4), pancolitis (extending from the cecum to the rectum and analogous to extensive UC; n = 5), or UC in remission (n = 5). CD and UC designations followed Vienna (Gasche et al., 2000) and Montreal (Silverberg et al., 2005) classifications, respectively. In no case was CD strictureing or penetrating. Tissue biopsy during endoscopy was performed by gastroenterologists who were experienced in the endoscopy of IBD patients and who collected one additional biopsy specimen for molecular analysis at a site adjacent to that routinely sampled for histopathologic study. Whenever active disease was present, inﬂamed regions were biopsied while avoiding regions with macroscopically ulcerated tissue. Such biopsy specimens for molecular analysis were taken from the terminal ileum in CD and from the mid-descending colon in UC (the latter to allow comparisons between patients with pancolitis and patients with exclusively left-sided colitis). Biopsy specimens for molecular analysis were snap-frozen in liquid nitrogen and stored at −80°C until further processing. Sample collection in adolescents was as in adults.

Histologic Analysis. Biopsy specimens taken for routine histologic examination were ﬁxed in 4% neutral buffered formaldehyde solution and embedded in parafﬁn. Sections were stained with hematoxylin and eosin. Experienced histopathologists scored ﬁndings in the sections using standard systems. CD and UC were diagnosed by standard criteria using clinical, endoscopic, radiologic, and histopathologic ﬁndings (data not shown) (Stange et al., 2008; Van Assche et al., 2010). Even though they had no symptoms, two IBD patients had histologically evident intestinal inﬂammation on routine surveillance colonoscopy. In four patients, histologic examination led to the initial diagnosis of IBD, and in 10 patients, IBD had ﬂared after a period of remission.

Total RNA Isolation, Reverse-Transcription, and Real-Time Polymerase Chain Reaction Studies. RNA was isolated from snap-frozen human biopsy specimens (TRIZol: Invitrogen, Vienna, Austria) and was subjected to reverse transcription (GeneAmp Gold RNA PCR Core Kit; Applied Biosystems, Foster City, CA) according to manufacturers’ instructions. Real-time polymerase chain reaction (RT-PCR) work was performed on a GeneAmp 7900 Sequence Detection System with GeneAmp 7900 SDS software (Applied Biosystems), using reverse-transcribed cDNA (3.5 μl of cDNA) in a 20-μl PCR reaction mixture containing 10.5 μl of SYBR Green Universal PCR Master Mix (Applied Biosystems) and 0.7 μl of each set of gene-speciﬁc primers. Thermal cycling conditions were as follows: 2 minutes 50°C, followed by 95°C for 10 minutes, 40 cycles at 95°C for 15 seconds, and 60°C for 1 minute. Reactions were performed in duplicate on 96-well plates and were repeated twice. At the end of each run, PCR reaction products were checked by gel electrophoresis for correct size and quality. Table 4 lists primer pairs for investigated hepatobiliary transporters, nuclear receptors, and detoxifying enzymes and for both glyceraldehyde 3-phosphate dehydrogenase, against which expression of other genes was normalized, and inducible nitric oxide synthase (iNOS), evaluated as a marker of inﬂammation.

Statistical Analysis

Each patient characteristic is presented as a median with a minimum and maximum. To compare patient groups with the control group, Mann–Whitney U tests were used. All results are shown with means ± S.D. Power analyses were used to calculate the minimum sample size required to prevent an underpowered study. Statistical analysis was performed with IBM SPSS Statistics software 19.0.0 (IBM SPSS, Armonk, NY).

Results

Crohn’s Disease

Changes in Ileal mRNA Expression Levels for BA Transporters and Detoxifying Enzymes. BA uptake transporter (ASBT) and BA enterocyte-apical efflux transporter (breast cancer–related protein; BCRP) mRNA expression levels were signiﬁcantly lower in inﬂamed regions in patients with CD ileitis (Figs. 1 and 2) than in controls, with levels of ASBT expression in CD ileitis 36% (P = 0.003) of those in control-patient ileal mucosa (Fig. 2A). A less pronounced but also signiﬁcant decrease was observed in CD patients in remission, in whom levels of ASBT expression were 53% (P = 0.03) of those in controls (Fig. 2B). In CD ileitis in adolescents, mRNA expression levels of ASBT were decreased (15% of controls; P < 0.001). In adolescents suffering from UC without backwash ileitis, ASBT expression levels were similar to those in controls (data not shown). The reduction in BCRP expression was signiﬁcant for patients with acute ileitis (57%; P < 0.05), but not for patients in remission (67%). Expression of all other BA transporters studied [a second enterocyte-apical efflux transporter, multidrug resistance protein 1 (MDR1); the intracellular ileal BA binding protein, which contributes to BA movement through enterocytes (Landrier et al., 2002); the basolateral export pumps heteromeric organic solute transporter αβ (OSTα/β) and multidrug resistance protein 3 (MRP3)] did not differ signiﬁcantly between healthy controls and patients with either active CD or CD in remission. Expression of the sulphating enzyme SULT2A1, a component of the ileal BA detoxification system, fell in active CD to levels 31% (P < 0.02) of those in normal mucosa. In remission, expression levels were equal to those in controls. As no conspicuous differences in mRNA expression levels were seen between CD subgroups (inﬂammation in the terminal ileum, ileo-terminal ileum, or ileocolon), analyses of samples from these sites were pooled.

Ulcerative Colitis

Changes in Colonic mRNA Expression Levels for BA Transporters and Detoxifying Enzymes. Downregulation of BA transporters in the descending colon was impressive in UC (Fig. 3). Significant differences in results between patients with pancolitis and patients with distal (left-sided) colitis permitted us to assess ﬁndings separately in these two groups. In pancolitis, mRNA expression levels for the basolateral efflux pumps MRP3 and OSTα/OSTβ fell signiﬁcantly to 50% and 20%/29% (all P < 0.04) of values for normal colon mucosa, respectively (Fig. 3A). Furthermore, the expression of two apical efflux pumps, MDR1 and MRP4, was also signiﬁcantly reduced to 17% and 58% of control values, respectively (both P < 0.03). We analyzed expression in the descending colon of the hydroxylating cytochrome P450 control enzyme CYP3A4, representative of the detoxication system, and observed in pancolitis a 1.4-fold rise (P = 0.012). In left-sided colitis as well as in remission, CYP3A4

<table>
<thead>
<tr>
<th>Inflammatory markers before colonoscopy</th>
</tr>
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<tbody>
<tr>
<td>Control (n = 9)</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
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<tr>
<td>Leukocytes (per μl)</td>
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</tbody>
</table>

*P < 0.05 compared with controls.
mRNA expression levels were less than those in healthy mucosa; however, these changes did not reach statistical significance (Fig. 3, B and C). In distal colitis, no significant decrease was measured, although all investigated mRNA expression levels trended downward (Fig. 3B). Finally, in patients with UC in remission, quantitative assessments revealed no significant changes in BA transporter mRNA expression levels compared with those in controls or in patients with active UC (Fig. 3C).

Changes in mRNA Expression Levels for Nuclear Receptors. To further explore potential changes in key regulatory nuclear receptors, we assessed expression of FXR, which is activated by BA (its natural ligands), of the pregnane X receptor (PXR) and of the vitamin D receptor (VDR), which can also be activated by BA. In patients with CD, both active and in remission, mRNA levels of FXR; of its target, the small heterodimer partner (SHP); and of PXR did not change significantly from those in controls (Fig. 2, A and B). Expression of VDR was reduced in patients with active CD but not significantly so. By contrast, in patients with UC and pancolitis, both VDR and PXR levels fell compared with controls (both \( P < 0.01 \)); FXR levels were again unchanged (Fig. 3A). In patients with left-sided colitis or with UC in remission, no nuclear receptor investigated showed decreased expression levels (Fig. 3, B and C).

Changes in mRNA Expression Levels for the Inflammatory Marker iNOS. We measured iNOS expression levels in all adult patients (Table 5). A 17-fold increase in iNOS levels compared with normal colon was measured in mucosa from patients with UC and pancolitis (\( P = 0.003 \)) and a 6-fold increase was measured in mucosa from patients with UC and left-sided colitis (not significant), but in mucosa from patients in remission, no significant change in iNOS expression was found. Active CD was characterized by a 27-fold increase versus normal mucosa (\( P < 0.001 \)); remission saw full resolution.

### TABLE 4

<table>
<thead>
<tr>
<th>Protein-Coding Gene Name</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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</thead>
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<tr>
<td>GAPDH</td>
<td>tctcctcttgacttcaacagcag</td>
<td>cccctgtgtcgttgaccaaatc</td>
</tr>
<tr>
<td>ASBT</td>
<td>gttacctctcgcctttcttctggtc</td>
<td>gactctgtgtatgccctgttcaacatag</td>
</tr>
<tr>
<td>MRP3</td>
<td>GCCACGCTCTCTTTGACA</td>
<td>attgacgacgtgaggatga</td>
</tr>
<tr>
<td>OSTα</td>
<td>caggttgcttgcccgtttc</td>
<td>attccgcctgcaacgtgtaatag</td>
</tr>
<tr>
<td>OSTβ</td>
<td>caggttgcttgcccgtttc</td>
<td>gacccgtccttgtaatccacatag</td>
</tr>
<tr>
<td>I-BABP</td>
<td>gcaggaagaaagcacaacatcag</td>
<td>gctctgttgcttggtgataag</td>
</tr>
<tr>
<td>BCRP</td>
<td>caggttgcttgcccgtttc</td>
<td>tccacatgttggaatgtcaag</td>
</tr>
<tr>
<td>MDR1</td>
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<td>FXR</td>
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<td>SHP</td>
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<td>cctctgtgagcaccacag</td>
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<td>PXR</td>
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<td>CYP3A4</td>
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<td></td>
</tr>
<tr>
<td>SULT2A1</td>
<td>ccacccctccctccatcagttatc</td>
<td>acaactcttggtgattaacaag</td>
</tr>
</tbody>
</table>

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; I-BABP, ileal bile acid binding protein; TNFs, tumor necrosis factor a.

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**Note:** Means ± SD are shown. *\( p < 0.05 \) compared to controls.

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**Fig. 2.** RT-PCR in CD. RT-PCR results; analysis of major ileal bile acid transporters in inflamed (CD ileitis) (A) and uninflamed (CD in remission) (B) ileal mucosa of patients suffering from CD and in controls. Expression values are shown relative to healthy controls (dotted line). Means ± S.D. are shown. *\( P < 0.05 \) compared with controls. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; I-BABP, ileal bile acid binding protein.
FGF-19. To explore potential changes in BA homeostasis in a younger patient collective without long-standing medical history, we started to measure expression levels in the ileum of adolescent IBD patients. In these therapy-naïve patients, we repeated assays of ASBT and FXR expression levels. All adolescents with CD had active ileitis, and all UC patients suffered from histologically typical pancolitis without ileal inflammation in terms of backwash ileitis (data not shown). Compared with controls without intestinal inflammation, FGF-19 mRNA expression levels were significantly lower in adolescents with CD ileitis (10%; \( P < 0.001 \)) and UC colitis despite the absence of backwash ileitis (34%; \( P < 0.001 \)) (Table 6). mRNA expression levels of ASBT (15%; \( P < 0.001 \)) and FXR (58%; \( P = 0.003 \)) were decreased in CD ileitis (data not shown).

**Discussion**

The overall aim of this study was to identify molecular changes in intestinal BA homeostasis in mucosal biopsy specimens from patients suffering from IBD of various phenotypes. We demonstrate that ileitis in CD influenced expression of selected BA transporters and metabolizing enzymes, whereas pancolitis in UC profoundly affected expression of BA transporters and regulatory nuclear receptors. Contrary to CD, molecular changes in UC were completely reversible during the remission phase.

The most striking observation in CD is the downregulation of expression of mRNA for the main intestinal BA uptake transporter, ASBT, during ileitis (Fig. 2A). This is in line with previous reports (Jung et al., 2004; Kwon and Carey, 2004; Wojtal et al., 2009); however, our data indicate that reductions in mRNA levels of ASBT mRNA persist during remission, consonant with increased fecal excretion of BA in patients with CD in clinical remission (Rutgeerts et al., 1979) and suggesting that alternative BA absorption mechanisms cannot compensate for reduced ASBT function (Dawson et al., 2003).

The underlying mechanism for reduced ASBT levels in CD is not fully understood. Sundaram et al. (1998) have shown in a rabbit model that in the chronically inflamed ileum, Na⁺-BA cotransport is inhibited by decreases in both cotransporter affinity for BA and cotransporter

![Fig. 3. RT-PCR in UC. RT-PCR results; analysis of most colonic bile acid transporters in pancolitis (A), left-sided colitis (B), and uninflamed colonic mucosa of patients with UC in remission (C) versus controls. Expression values are expressed relative to healthy controls (dotted line). Means ± S.D. are shown. *\( P < 0.05 \) compared with controls. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.](image-url)
numbers; specific but undetected immune-inflammatory mediators were invoked. A large body of evidence exists for cytokine-dependent deregulation of hepatobiliary BA transporters during inflammation (Geier et al., 2005). Therefore, we paid attention to biopsy in active-disease–inflamed regions. Although we found elevated levels of the acute-phase reactant CRP in serum of our patients with ileitis, since two transporters were selectively downregulated, the mechanism of downregulation seems different from that of acute-phase reaction in the liver. Genome sequencing in healthy persons revealed multiple functionally relevant variants in SLC10A2, encoding ASBT, that may influence BA homeostasis (Ho et al., 2011). SLC10A2 affects susceptibility to cholelithiasis (Renner et al., 2009). Analyses of mutations of SLC10A2 in IBD patients have not yet been published. We expected discriminable ASBT expression levels between operated and not-operated patients. However, this was not the case. The neoterminal ileum seems to adopt the BA transport capacity of the missing terminal ileum.

Via FXR, BA can selectively decrease transcription of human ASBT and its target SHP (Neimark et al., 2004). We found no change in FXR and SHP expression levels in adults; however, our study was limited by the nonavailability of measurements of FXR concentrations of protein or of FXR activity. Repeated measurements of FXR expression levels in therapy-naïve adolescents suffering from CD ileitis revealed—in contrast to adult patients—significant abnormalities. Certain findings by other teams argue for an FXR-dependent deregulation of hepatobiliary BA transporters during inflammation (Ejderhamn et al., 1991; Gnewuch et al., 2009). All measured BA transporter mRNA levels were downregulated in biopsy specimens from patients with pancolitis (Fig. 3A). Interestingly, these findings differ from those in patients with left-sided colitis, in whom no significant downregulation occurred (Fig. 3B). This may be attributed to a lesser inflammatory response, as reflected by lower CRP values. Although differences in CRP levels between control patients and patients with CD ileitis or UC pancolitis were minor, they achieved statistical significance. As described (Saverymuttu et al., 1986), CD was associated with a stronger CRP response than was UC. Whereas ASBT mRNA levels were persistently low during remission in CD, no significant changes were found during remission in UC (Fig. 3C). On microscopy, material from patients with pancolitis and patients with left-sided colitis differed principally in the degree of inflammation present (data not shown). Patients with IBD have a disturbed balance between pro- and anti-inflammatory cytokines in the mucosa, one possible reason why antiinflammatory treatment is efficient (Rogler and Andus, 1998), and the production of proinflammatory cytokines is higher in severe UC colitis than in colitis with lesser endoscopic grades of inflammation (Ishiguro, 1999; Raddatz et al., 2005). In our study, mRNA expression levels of iNOS, which in pancolitis is expressed after induction by certain cytokines, microbes, and bacterial products (Kofios et al., 2004), were significantly increased (15-fold), higher than in left-sided colitis (Table 5). During inflammation in the liver, several nuclear hormone receptors are downregulated by inflammatory cytokines, leading to downregulation of their target transporter and detoxification genes (Beigneux et al., 2000; Kim et al., 2003). Our results in pancolitis permit the hypothesis that—as in the liver—markedly increased levels of cytokines during pancolitis mediate disturbances in the expression of regulatory nuclear receptors such as PXR or VDR that consequently are manifest in changes in levels of mRNA of colonic BA transporters, both apical (MRP4, MDR1) and basolateral (MRP3, O斯塔β)

TABLE 5
<table>
<thead>
<tr>
<th>Relative Absolute</th>
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<tbody>
<tr>
<td>%</td>
</tr>
<tr>
<td>UC</td>
</tr>
<tr>
<td>Controls</td>
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<td>Pancolitis</td>
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<td>Controls</td>
</tr>
<tr>
<td>Ileitis</td>
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<td>Remission</td>
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*P < 0.05 compared with controls (Student’s t test).

Vavassori et al. (2009) showed that, as a component of a network of nuclear receptors, FXR regulates intestinal innate immunity and homeostasis. In vitro assays show that intestinal inflammation strongly reduces FXR activation (Gadaleta et al., 2011a). We speculate that long histories of intestinal inflammation and therapy lead to adaptation of the FXR expression levels in CD.

In CD ileitis, we also observed downregulation of the enteroocyte-apex BA export transporter BCRP and of the detoxifying enzyme SULT2A1 (Fig. 2A). SULT2A1 catalyzes the formation of BA sulfates within enterocytes (Alnouti, 2009). We speculate that reduced ASBT levels lead to diminished intracellular amounts of BA; compensation for this reduces BA sulphation and BA apical efflux. In disease remission, persistently reduced ASBT mRNA levels seem sufficient to maintain physiologic BA homeostasis because mRNA levels of all other investigated BA transporters are comparable with those in controls (Fig. 2B). The lack of change in FXR levels could, in particular, indirectly argue for balanced intracellular BA concentrations in long-standing CD; however, these hypotheses are only speculative.

Not only CD but also severe UC is well known to be associated with decreased serum levels of BA and increased fecal levels of BA (Ejderhamn et al., 1991; Gnewuch et al., 2009). All measured BA transporter mRNA levels were downregulated in biopsy specimens from patients with pancolitis (Fig. 3A). Interestingly, these findings differ from those in patients with left-sided colitis, in whom no significant downregulation occurred (Fig. 3B). This may be attributed to a lesser inflammatory response, as reflected by lower CRP values. Although differences in CRP levels between control patients and patients with CD ileitis or UC pancolitis were minor, they achieved statistical significance. As described (Saverymuttu et al., 1986), CD was associated with a stronger CRP response than was UC. Whereas ASBT mRNA levels were persistently low during remission in CD, no significant changes were found during remission in UC (Fig. 3C). On microscopy, material from patients with pancolitis and patients with left-sided colitis differed principally in the degree of inflammation present (data not shown). Patients with IBD have a disturbed balance between pro- and anti-inflammatory cytokines in the mucosa, one possible reason why anticytokine treatment is efficient (Rogler and Andus, 1998), and the production of proinflammatory cytokines is higher in severe UC colitis than in colitis with lesser endoscopic grades of inflammation (Ishiguro, 1999; Raddatz et al., 2005). In our study, mRNA expression levels of iNOS, which in pancolitis is expressed after induction by certain cytokines, microbes, and bacterial products (Kofios et al., 2004), were significantly increased (15-fold), higher than in left-sided colitis (Table 5). During inflammation in the liver, several nuclear hormone receptors are downregulated by inflammatory cytokines, leading to downregulation of their target transporter and detoxification genes (Beigneux et al., 2000; Kim et al., 2003). Our results in pancolitis permit the hypothesis that—as in the liver—markedly increased levels of cytokines during pancolitis mediate disturbances in the expression of regulatory nuclear receptors such as PXR or VDR that consequently are manifest in changes in levels of mRNA of colonic BA transporters, both apical (MRP4, MDR1) and basolateral (MRP3, O斯塔β)

TABLE 6
<table>
<thead>
<tr>
<th>Relative Absolute</th>
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<tr>
<td>%</td>
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<tr>
<td>UC (pancolitis)</td>
</tr>
<tr>
<td>CD (ileitis)</td>
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*P < 0.05 compared with controls (Student’s t test).
Reduced colonic BA transport and metabolism could lead to concentrations of intraluminal BA >3 mmol, resulting in diarrhea. Moreover, increased colonic BA concentrations appear associated with enhanced mucosal permeability and structural changes (Corazza et al., 1979) that may additionally contribute to diarrhea in patients with UC. Intraluminal BAs are considered as tumor promoters in colorectal carcinogenesis; interestingly, IBD patients have an increased risk of colorectal dysplasia and cancer (Torres et al., 2011). Furthermore, various xenobiotics—including some glucocorticoids or immunosuppressants—share intestinal transporters with BA (e.g., MDR1) (Luo et al., 2004), and the downregulation of these transporters during pancolitis that we observed may have undefined consequences for the metabolism of administered drugs. On the other hand, in our cohort, we could not find a correlation between medications used and the changes in expression observed, permitting the inference that these agents influence BA transporter expression levels only slightly. However, proof of this must be obtained in treatment-naive IBD patients.

BAM in IBD is not fully understood. Patients suffering from primary BAM (as with ASBT mutation) are not able to sufficiently reabsorb BA in the gut; diarrhea-predominant irritable bowel syndrome (IBS) patients have BAM with high intraluminal BA levels due to missing FGF-19–mediated repression of hepatic BA biosynthesis (Walters et al., 2009). Depending on their concentrations within the colonic lumen, BA can have, via FXR signaling, not only a prosecretory but also an antisecretory effect (Keating et al., 2009). Intraluminal BA in moderate quantities can prevent fluid accumulation in the intestine by inhibition of the Na+ channel and K+-ATPase (Mroz et al., 2004; expression of both transporters was described as decreased in UC colitis (Greig et al., 2004; Sullivan et al., 2009). Our studies in adolescent IBD reveal low ideal FGF-19 levels in CD and UC. Summing up, in CD ileitis, BA reabsorption seems to be reduced due to low ASBT levels; in UC pancolitis, BA transport seems to be disturbed due to (inflammation-caused) transporter expression downregulation independent of the presence of backwash ileitis. In both cases, hepatic BA synthesis is enhanced due to missing FGF-19 signaling, and the antisecretory effect of BA disappears. These observational converges in the conclusion that BA in pathologic intraluminal concentrations may induce the onset of diarrhea in IBD. Our findings make clear that BA handling in IBD is altered, and suggest that targeted medical correction of alterations in BA transport and metabolism could be a new therapeutic approach to IBD.

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