Phospholipase A₂ of Microbiota as Pathogenetic Determinant to Induce Inflammatory States in Ulcerative Colitis: Therapeutic Implications of Phospholipase A₂ Inhibitors

Wolfgang Stremmel Simone Staffer Nicole Stuhmann
Hongying Gan-Schreier Annika Gauss Nina Burger Daniel Hornuss

Department of Internal Medicine IV, University Clinics of Heidelberg, Heidelberg, Germany

Keywords

Intestine-specific kindlin 2 knockout mouse · Mucosal inflammation · Mucus · Phospholipase A₂ inhibitors · UDCA-LPE

Abstract

Background: Attack by commensal microbiota is one component of induction of inflammatory episodes in ulcerative colitis (UC). In UC, the mucus layer is intrinsically devoid of phosphatidylcholine (PC) resulting in low hydrophobicity which facilitates bacterial invasion. Colonic ectophospholipase-carrying bacterial strains are likely candidates to further thinning the PC mucus barrier and to precipitate inflammatory episodes. **Objective:** To evaluate the effect of phospholipase A₂ (PLA₂) inhibitors on inflammation in a genetic UC mouse model. **Methods:** As PLA₂ inhibitor, we applied the bile acid-phospholipid conjugate ursodeoxycholate-lysophosphatidylethanolamide (UDCA-LPE) or as control 5% Tween 80 by oral gavage to intestine-specific kindlin 2 knockout mice. **Results:** Luminal UDCA-LPE reduced the PLA₂ activity in stool by 36 ± 8%. Concomitantly no inflammatory phenotype was observed when compared to kindlin 2^(-/-) mice not treated with UDCA-LPE. The improvement was

documented in regard to stool consistency, calprotectin levels in stool, and macroscopic/endoscopic as well as histologic features of the mucosa. The pattern of colonic microbiota distribution obtained in the UC phenotype mice was reversed by UDCA-LPE to the control mice pattern. **Conclusion:** The inhibition of the bacterial ectophospholipase A₂ activity improves mucosal inflammation in a genetic mouse model of UC. It is assumed that the remaining mucus PC shield is better preserved when luminal PLA₂ is suppressed.

© 2018 The Author(s)

Published by S. Karger AG, Basel

Introduction

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) of unknown etiology, for which only symptomatic anti-inflammatory therapy is available. A defective mucosal barrier is postulated as one possible pathogenetic mechanism [1]. Indeed in patients with UC, low intrinsic mucus phosphatidylcholine (PC) content (reduction by 70% of control levels) even in remission has been documented [2, 3], which reduces surface hydrophobicity [4] and, thus, facilitates commensal bacteria to invade and induce inflammation [5]. PC is mainly secret-

ed in the ileum and only marginally in the colon [6]. It was shown that PC is translocated from systemic sources across tight junctions (TJ) to the luminal side of mucosal cells driven by a negative electrochemical gradient generated by the cystic fibrosis transmembrane conductance regulator (CFTR) [7]. After TJ translocation, PC is bound to membrane-associated mucin 3, from where it is handed to secretory mucin 2 [5, 8]. The PC-mucin 2 complex moves within the upper mucus layer along the intestinal wall distally to the colon and rectum to provide sufficient hydrophobic protection against colonic microbiota.

Paracellular PC transport to the mucus was prohibited by TJ disruption in genetic mouse models with tamoxifen-sensitive intestinal deletion of kindlin 1 and 2 [5] with consequent induction of a UC phenotype, due to impaired surface hydrophobicity facilitating the invasion of commensal bacteria [5].

As shown in human UC patients in remission, as little as 30% remaining mucus PC content may be sufficient to maintain a hydrophobic barrier [2, 3]. Acute inflammatory episodes occur when PC is further reduced below a critical level. This is most likely caused by a colonic microbiota with enhanced ectophospholipase activity. The pathogenetic potential of such bacterial species is known from *Helicobacter pylori*, which carries an ectophospholipase to break the gastric mucus PC barrier for invasion and colonization of the surface of gastric mucosal cells [9]. In the gut, we postulate that the conversion to an ectophospholipase-rich microbiota by changes of the hormonal/metabolic state or by systemic inflammatory stress conditions is capable of further reducing PC-mediated hydrophobicity below a critical threshold which opens the mucus barrier for invasion by the full spectrum of commensal bacteria, resulting in mucosal inflammation. Because of its extended exposure with phospholipase-containing bacteria, the rectum is prone to mucosal inflammation in UC with its intrinsic mucus PC depletion. From there, the colitis extends to upper colonic segments depending on the degree of mucus PC reduction and thus, enhanced susceptibility of the mucosa to bacterial invasion. Thus, the hypothesis of a double-edged sword pathogenesis of UC was generated: (1) an intrinsic, genetically inherited low mucus 2-bound PC content, which reduces protective surface hydrophobicity but, per se, does not induce inflammation; (2) reduction of mucus PC level below a critical level by enhanced ectophospholipase activity of the microbiota allowing further bacterial invasion and consequent inflammation.

Therefore, therapeutically two concepts can be employed: (1) supplementation of PC to mucin 2 by de-

layed-released oral PC application to fill the gaps and reestablish a fully operative hydrophobic barrier. It serves for the prevention of acute inflammatory episodes and is an ideal therapy for maintenance of clinical remission. (2) Inhibition of the microbiota ectophospholipase activity as pathogenicity factor to allow mucus and mucosa bacterial invasion with consequent inflammation. This predominant anti-inflammatory concept is therefore meant for therapy of inflammatory states of UC as well as any other IBD. It may even function when the mucus is destroyed as a consequence of inflammation. As nontoxic, poorly absorbable phospholipase A₂ (PLA₂) inhibitor, we chose the phospholipid-bile acid conjugate ursodeoxycholate-lysophosphatidylethanolamide (UDCA-LPE), which in previous studies was shown to be effective in the liver [10]. The aim of the present study was to prove this hypothesis. As experimental animal model we chose intestine-specific kindlin 2^(-/-) mice kept under non-germ, free conditions [11, 12]. This is an in-house generated genetic mouse model with disrupted lateral tight junctions which causes an UC phenotype [5].

Materials and Methods

Induction of a Mouse Colitis Phenotype and Its Prevention

In this non-human-based study we employed intestine-specific kindlin 2^(-/-) mice and handled them as described recently [5] (online suppl. material; for all online suppl. material, see www.karger.com/doi/10.1159/000486858).

For the induction of the phenotype, female control wild-type and mutant mice (12 weeks old, body weight of 31 ± 2 g) were intraperitoneally injected with 0.2 mg tamoxifen daily for up to 3 days before being sacrificed 1 day later. Western blotting of isolated mucosal cells [5, 13] obtained from kindlin 2^(-/-) mice verified depletion of the targeted kindlin 2 in comparison to wild-type controls [5]. As PLA₂ inhibitor we chose UDCA-LPE (Chemi, Milan, Italy) which in vivo is degraded to UDCA and LPE [14]. Over the time of tamoxifen injection, the kindlin 2^(-/-) mice were daily orally gavaged (tube of 6.5 cm in length, 9918, Codence Science, Cranston, RI, USA) either with a 100- μ L bolus of 5% Tween 80 (control) or with 50 mg/kg UDCA-LPE in 5% Tween 80 ($n = 6$ mice for each group) [5].

Analysis of Absorption Capacity of UDCA-LPE in vitro and in vivo

For in vitro determination of transcellular movement of UDCA-LPE (apical application of 100 μ M in PBS), the confluent and polarized 21-day cultured intestinal tumor cell line CaCo2 in transwell culture dishes was employed with a transmembrane electrical resistance (TER) of >300 [7]. The basal recovery of UDCA-LPE over 1 h was determined by ESI-MS/MS [7].

For in vivo UDCA-LPE recovery studies wild-type mice were starved overnight before administration of a 100- μ L bolus of 50

Fig. 1. Endoscopic and histologic features of the ileum in wild-type mice (**a**) kindlin 2^(-/-) mice orally gavaged with Tween 80 only (**b**) and kindlin 2^(-/-) mice orally gavaged with UDCA-LPE in Tween 80 (**c**) over the 3 days of tamoxifen exposure (scale bars = 25 μ m).

mg/kg UDCA-LPE. After 4 h, the mice were sacrificed and UDCA-LPE was determined by ESI-MS/MS in serum, liver homogenate, and collected luminal content of the intestine.

Clinical Evaluation of the UC Activity

After 3 days of tamoxifen treatment, ileal endoscopy was retrogradely performed as described in detail [5]. Endoscopic alterations were evaluated by the modified MEICS score [15]. In addition, ileum and colon were excised and analyzed by the macroscopic and histological colitis score [16] (online suppl. material). The calprotectin concentration in stool was determined as a marker of mucosal inflammation [17, 18] using the S100A9/calprotectin, mouse, ELISA kit (Hycult Biotech, Uden, The Netherlands).

Impact of UDCA-LPE on Microbiota

We analyzed first the PLA₂ activity in stool under control wild-type, colitis and additional UDCA-LPE pretreatment conditions [10, 19].

Microbiota analysis was performed by bacterial 16S rRNA-sequencing (variable V3-V6 region) of the collected stool samples (MicroBIOMix GmbH, Regensburg, Germany) (online suppl. material).

Statistical Analysis

Each experiment was repeated 6-fold. Statistical analysis was performed using Prism 4.0 software (GraphPad Software Inc., La Jolla, CA, USA). Differences between groups were evaluated using the Mann-Whitney U test. Multiple groups were compared by one-way ANOVA with a Dunnett post hoc test. For the microbiota analysis, the statistical software SPSS was used (IBM Corp., Armonk, NY, USA). Data are presented as means $\pm$ SD or medians with range, and $p < 0.05$ was considered statistically significant.

Results

Previously it was shown that UC is due to an intrinsic low PC content of the mucus, which impairs surface hydrophobicity and allows microbiota invasion followed by mucosal inflammation [2–5]. PC passes from systemic sources along a paracellular route between enterocytes across the TJ barrier to the luminal side for incorporation

Fig. 2. Colonic weight (including stool), colonic length, and calprotectin concentration in stool of wild-type control, in kindlin 2^(-/-) mice injected with tamoxifen and orally gavaged with Tween 80 only, and in kindlin 2^(-/-) mice injected with tamoxifen and concomitantly orally gavaged with UDCA-LPE in Tween 80 (means $\pm$ SD, * $p < 0.05$).

Table 1. Macroscopic and histopathological evaluation of the inflammatory phenotype in wild-type and kindlin 2^(-/-) mice orally gavaged with 5% Tween 80 only or UDCA-LPE in Tween 80 over the 3 days of tamoxifen exposure

	Wild-type	Kindlin 2 ^(-/-)	Kindlin 2 ^(-/-) + UDCA-LPE
<i>Macroscopic colitis score</i>			
Stool score ^a	1 (0–2)	11 (10–13)	5 (5–5)
Colon weight and fecal content, g	0 (0–0)	4 (3–5)	2 (1–3)
Colon weight score ^b	0.23 $\pm$ 0.02	0.45 $\pm$ 0.04	0.25 $\pm$ 0.05
Colon length, cm	0 (0–0)	4 (4–4)	3 (2–3)
Colon length score ^c	4.57 $\pm$ 0.53	3.27 $\pm$ 0.39	4.67 $\pm$ 0.37
<i>p</i> (total score vs. wild-type)	1 (0–2)	3 (2–4)	0 (0–1)
<i>p</i> (total score vs. no UDCA-LPE)		0.050	0.037
<i>Histopathological colitis score</i>			
Extent of inflammation ^d	0 (0–0)	10 (9–10)	2 (1–3)
Crypt architecture ^e	0 (0–0)	2 (1–3)	0 (0–1)
Hyperemia/edema ^f	0 (0–0)	2 (2–3)	1 (1–2)
Immunocyte infiltration ^g	0 (0–0)	3 (2–3)	0 (0–1)
<i>p</i> (total score vs. wild-type)	0 (0–0)	3 (2–3)	0 (0–1)
<i>p</i> (total score vs. no UDCA-LPE)		0.034	0.046
<i>Major colitis score</i>			
(macroscopic and histopathological score)	0 (0–2)	20 (20–23)	7 (6–8)
<i>p</i> (total score vs. wild-type)		0.046	
<i>p</i> (total score vs. no UDCA-LPE)			0.046

The data are shown as the mean $\pm$ SD or median (range). ^a 0 = normal, 1 = loosely shaped, 2 = amorphous, sticky, 3 = diarrhea, +1 for blood. ^b 0 $\leq$ 10%, 1 = 10–50%, 2 $\geq$ 50–100%, 3 $\geq$ 100–150%, 4 $\geq$ 150% weight gain compared to the wild-type mean. ^c 0 $\leq$ 5%, 1 = 5–14%, 2 = 15–24%, 3 = 25–35%, 4 $\geq$ 35% shortening compared to the wild-type mean. ^d 0 = no inflammation, 1 = mucosa, 2 = mucosa and submucosa. ^e 0 = intact, 1 = regenerative, 2 = destroyed. ^f 0 = none, 1 = discrete, 2 = moderate, 3 = severe. ^g 0 = none, 1 = discrete, 2 = moderate, 3 = severe.

Fig. 3. Effect of UDCA-LPE on PLA₂ activity in mouse stool (means $\pm$ SD, * $p < 0.05$, ** $p < 0.01$).

into mucus [7]. When the PC translocation mechanism is disturbed, UC can develop [5]. The predisposition to inflammatory episodes is enhanced by the phospholipase activity of the microbiota because of the further thinning of the mucus PC layer. The rationale of this study was to prove this hypothesis.

Intestinally Deleted Kindlin 2 Mice Reveal an UC Phenotype

As model we chose adult mice with tamoxifen-inducible, villin-Cre-mediated intestinal deletion of kindlin 2, which is known to activate integrin β_1 as control instance of TJ stabilization [11, 12]. Western blot analysis of isolated mucosal cells in mutants revealed the completeness of the kindlin 2 knockout and a decrease in representative proteins constituting the link from kindlin 2 to integrin β_1 activation, to adherence junctions (e-cadherin), and finally to TJs (ZO1, occludin, claudin 2) [5]. Preceding freeze-fracture electron microscopy studies [5] showed disturbed TJs which occurred already in the preinflammatory state after 2 days of tamoxifen exposure of the mutant mice. In the present study, also a general morphometric light microscopy analysis of mucosal biopsies after HE staining was performed (Fig. 1). The crypt luminal diameter (ratio of crypt lumen to total crypt diameter) in the ileum (at the same position within the crypt) of wild-type mice was 0.082 ± 0.021 and in kindlin 2^(-/-) mice 0.162 ± 0.036 and in kindlin 2^(-/-) mice with concomitant UDCA-LPE application 0.187 ± 41 . While kindlin 2^(-/-) mutants had enlarged lumina compared to wild-type mice ($p < 0.01$), the treatment with UDCA-LPE had no impact on the diameter ($p > 0.05$) (Fig. 1).

The disturbed TJ barrier due to kindlin 2 deletion results in impaired luminal PC secretion with the pathophysiologic consequences of UC development [5]. Indeed, as also demonstrated in the present study, after 3 days of tamoxifen exposure, intestinally deleted kindlin 2^(-/-) mice expressed full-blown UC phenotype which was not observed in control mice exposed to tamoxifen (Fig. 1). The stool was soft, amorphous, sticky, and contained blood. The severe inflammatory phenotype of kindlin 2^(-/-) mice appeared with an opaque, thickened granular mucosa, absent vascular pattern with visible fibrin adherence, ulcerations and hemorrhagic lesions. Kindlin 2^(-/-) mice exhibited a significantly higher MEICS score compared to the concomitantly UDCA-LPE-treated mice (13.3 ± 1.7 vs. 1.8 ± 1.3 ; $p < 0.001$) compared to control wild-type mice (1.3 ± 0.6 ; $p > 0.05$). The edema of the intestinal wall resulted in colonic weight gain of $202.2 \pm 38.7\%$ and length reductions by $32.3 \pm 11.3\%$ in kindlin 2^(-/-) mice, respectively, versus control wild-type mice ($p < 0.05$ for both parameters; $n = 6$; Fig. 2; Table 1). In mutants, histopathology after HE staining revealed destroyed crypt architecture, mucosal and submucosal inflammation with infiltration of neutrophils and lymphocytes, ulcerations, and edema (Fig. 1; Table 1).

We postulated that an intrinsic low mucus PC constitutes a risk factor for bacterial invasion [20]. Accordingly, predominance of ectophospholipase carrying bacteria in stool can critically reduce the PC barrier. Indeed, the PLA₂ activity in stool was increased, either due to lack of PC or, more likely, due to changes of luminal environment or the applied stress for the animals (Fig. 3). Thus, inhibition of PLA₂ could be of therapeutic benefit.

UDCA-LPE as Poorly Absorbable PLA₂ Inhibitor

To ensure that UDCA-LPE as nontoxic PLA₂ inhibitor [14] exerts its activity mainly on the luminal microbiota, we examined the 1-h absorption capacity in vitro employing polarized CaCo2 cells as well as in vivo by determination of its concentration in stool, serum and liver 4 h after oral gavage application. The in vitro absorption capacity was $5.3 \pm 2.4\%$ per hour. In vivo, 4 h after oral gavage, $85.0 \pm 20.7\%$ of the UDCA-LPE was recovered in the intestinal lumen, whereas in total serum only $14.8 \pm 7.7\%$ and in total liver $0.75 \pm 0.11\%$ of applied UDCA-LPE were recorded. The experiments indicate that absorption of orally applied UDCA-LPE to the system is poor and the bulk of UDCA-LPE stays in the gut lumen. The postulated action of UDCA-LPE as PLA₂ inhibitor in stool was verified in stool samples of control mice. Incubation of 1 g mouse

Fig. 4. Effect of UDCA-LPE on the diversity of bacterial phyla in mouse stool without and with UDCA-LPE application in Tween 80 by oral gavage to kindlin 2^(-/-) mice in comparison to controls. The measured percentage distribution at the top is illustrated by cake diagrams at the bottom (means $\pm$ SD, * $p < 0.05$).

stool with 100 μ M UDCA-LPE over 1 h reduced the PLA₂ activity by $34 \pm 7\%$ ($n = 6$; $p < 0.001$).

Efficacy of UDCA-LPE on Stool PLA₂ Activity and Clinical Presentation in Mutant Mice with an UC Phenotype

Assuming that the commensal colonic bacteria contain ectophospholipases which are activated by stress conditions with consequent further reduction of the mucus PC content, we treated the kindlin 2^(-/-) mice by oral gavage with the poorly absorbed PLA₂ inhibitor UDCA-LPE [10, 14]. Indeed, PLA₂ activity in stool could be reduced compared to nontreated and even the wild-type mice (Fig. 3). Also demonstrated was the prevention of colitis, which was confirmed by ileoscopy, the macroscopic and histologic features described by the major colitis score (Fig. 1, 2; Table 1). Moreover, as most sensitive inflammatory read-out for intestinal inflammation the calprotectin concentration in stool was determined [18]. The value dropped from 2.101 ± 0.276 to 1.172 ± 0.341

mg/g stool in UDCA-LPE-treated mutants (wild-type control 0.976 ± 0.225 mg/g stool) ($p < 0.05$).

Impact of UDCA-LPE on Intestinal Microbiota

A comparison of the colonic microbiota of kindlin 2^(-/-) mice with and without UDCA-LPE treatment showed that mice without treatment experienced an expanded quantitative distribution of the various phyla, which was not observed in mice with UDCA-LPE. Indeed, the microbiota observed in the presence of UDCA-LPE was identical to that observed in control wild-type mice (Fig. 4; online suppl. Table S1). The most significant change was observed in regard to bacteroides. These phyla are considered as a cytoprotective microbiota [21], which is suppressed in mutant mice with a colitis phenotype and reversed to control levels by UDCA-LPE pretreatment. In addition, the S24-7 bacterial family was significantly reduced in the mutant mice and reversed to control values by UDCA-LPE ($p < 0.05$). All of the remaining bacterial phyla remained within the control range (Table 1).

Discussion

A new option for UC treatment targets the replenishment of the genetically determined depleted mucus PC content by oral application of a delayed-released PC preparation [22]. It was shown to restore the mucus PC content, which reestablished the barrier against microbiotic invasion and, thus, fighting mucosal inflammation [5, 22–24]. Assuming that genetically low mucus PC content is capable of maintaining barrier function up to a borderline concentration of 30% compared to normal subjects [2, 20], it could be postulated that a drop below this critical level allows bacterial invasion with consequent mucosal inflammation. Accordingly, inflammatory episodes in UC require an impaired mucosal barrier – low mucus PC content – and the invasion of bacteria: the double-edged sword for the pathogenesis of UC. Bacterial invasion requires threshold PC levels to be broken, which can be accomplished by ectophospholipase-containing bacteria. Consequently, the commensal stool flora can invade and cause inflammation.

The ectophospholipase-containing group of the gut microbiota species is not well defined and was not further evaluated in this study. However, it would be interesting to evaluate whether it is a feature of few specific species or abundant in most of the species with different levels of activity. Also of interest is whether specific microbiota – known to be of therapeutic value (e.g., *Escherichia coli* Nissle) – have low ectophospholipase activity and are able to displace those species with high enzymatic abundance. In our study, we observed a suppression of bacteroides and S24-7 bacteria together with an increase in stool phospholipase activity in the inflammatory state of the colitis phenotype. It is likely that they are secondarily suppressed by the applied stress for the animals, thus leaving space for more aggressive phospholipase-rich microbiota. However, when PLA₂ is inhibited by the nontoxic, poorly absorbed UDCA-LPE, both species reverse to normal distribution. The PLA₂ activity of total microbiota is significantly reduced, thereby mitigating the pathogenetic potential of bacterial-induced mucus PC consumption, and therefore the consequent mucus invasion by the commensal microbiota leading to mucosal inflammation. Accordingly, colitis/ileitis was significantly abrogated.

The concept of manipulating the microbiota for prevention/treatment of acute episodes of IBDs represents a defensive and rather safe therapeutic strategy. It is already supported by application of the topical acting antibiotic rifaximin which revealed impressive clinical improvement in Crohn's disease [25]. In UC, a dual therapeutic strategy

could be considered: (1) increasing the mucus barrier by supplementation of a delayed-released PC preparation, as maintenance of remission and (2) for inflammatory states the application of a PLA₂ inhibitor such as UDCA-LPE to induce remission. Indeed reducing the pathogenetic potential of the gut microbiota by UDCA-LPE or rifaximin is supported by the observation that UC does not occur without the gut microbiota, a phenomenon which is indeed proven in many genetic IBD models under germ-free conditions [26, 27]. The principle anti-inflammatory activity of PLA₂ inhibitors could also be utilized for other inflammatory diseases of the intestine. Therefore, we plan to test UDCA-LPE also in future mouse colitis models.

UDCA-LPE is an example of an intestinally working luminal PLA₂ inhibitor. Whether other phospholipases (C or D) may also be of relevance has yet to be determined. It is a first in class antimicrobial directed PLA₂ inhibitor with absent toxicity. Derivatives of this bile acid-lysophospholipid compound could be equally effective. There may be other structurally different PLA₂ inhibitors, but their potential toxicity after absorption has to be considered.

In this study, the proof of principle was supported, i.e., that luminally acting PLA₂ inhibitors may be of potential therapeutic use for intestinal inflammation by reduction of the pathogenetic ectophospholipases of microbiota.

Acknowledgement

We thank R. Faessler (MPI, Munich) for providing the conditional kindlin 2 knockout mice and F. Lasitschkaa (Department of Pathology, Heidelberg) for biopsy preparation.

Statement of Ethics

Animal studies followed the “ARRIVE” guidelines and were approved by the Heidelberg ethical committee (ref. No. 35-9185.81/6123/10 and 6284/11). This is a non-human-based study.

Disclosure Statement

The authors have no competing interest. W.S. is the inventor of the use of UDCA-LPE as an antimicrobial drug in the intestine. The University of Heidelberg holds this patent.

Funding Sources

This study was supported by the German Research Foundation (ref. No. STR 216/15-4).

References

- Podolsky DK: Inflammatory bowel disease. *N Engl J Med* 2002;347:417–429.
- Ehehalt R, Wagenblast J, Erben G, Lehmann WD, Hinz U, Merle U, et al: Phosphatidylcholine and lysophosphatidylcholine in intestinal mucus of ulcerative colitis patients. A quantitative approach by nanoElectrospray-tandem mass spectrometry. *Scand J Gastroenterol* 2004;39:737–742.
- Braun A, Treede I, Gotthardt D, Tietje A, Zahn A, Ruhwald R, et al: Alterations of phospholipid concentration and species composition of the intestinal mucus barrier in ulcerative colitis: a clue to pathogenesis. *Inflamm Bowel Dis* 2009;15:1705–1720.
- Braun A, Schonfeld U, Welsch T, Kadmon M, Funke B, Gotthardt D, et al: Reduced hydrophobicity of the colonic mucosal surface in ulcerative colitis as a hint at a physicochemical barrier defect. *Int J Colorectal Dis* 2011;26:989–998.
- Stremmel W, Staffer S, Schneider MJ, Gan-Schreier H, Wannhoff A, Stuhmann N, et al: Genetic mouse models with intestinal-specific tight junction deletion resemble an ulcerative colitis phenotype. *J Crohns Colitis* 2017;11:1247–1257.
- Ehehalt R, Jochims C, Lehmann WD, Erben G, Staffer S, Reininger C, et al: Evidence of luminal phosphatidylcholine secretion in rat ileum. *Biochim Biophys Acta* 2004;1682:63–71.
- Stremmel W, Staffer S, Gan-Schreier H, Wannhoff A, Bach M, Gauss A: Phosphatidylcholine passes through lateral tight junctions for paracellular transport to the apical side of the polarized intestinal tumor cell-line CaCo2. *Biochim Biophys Acta* 2016;1861:1161–1169.
- Korytowski A, Abuillan W, Amadei F, Makky A, Gumiero A, Sinning I, et al: Accumulation of phosphatidylcholine on gut mucosal surface is not dominated by electrostatic interactions. *Biochim Biophys Acta* 2017;1859:959–965.
- Mauch F, Bode G, Ditschuneit H, Malfertheiner P: Demonstration of a phospholipid-rich zone in the human gastric epithelium damaged by *Helicobacter pylori*. *Gastroenterology* 1993;105:1698–1704.
- Stremmel W, Staffer S, Wannhoff A, Pathil A, Chamulitrat W: Plasma membrane phospholipase A₂ controls hepatocellular fatty acid uptake and is responsive to pharmacological modulation: implications for nonalcoholic steatohepatitis. *FASEB J* 2014;28:3159–3170.
- Montanez E, Ussar S, Schifferer M, Bosl M, Zent R, Moser M, et al: Kindlin-2 controls bidirectional signaling of integrins. *Genes Dev* 2008;22:1325–1330.
- Meves A, Stremmel C, Gottschalk K, Fassler R: The Kindlin protein family: new members to the club of focal adhesion proteins. *Trends Cell Biol* 2009;19:504–513.
- Dawson JR, Bridges JW: Xenobiotic metabolism by isolated intestinal epithelial cells from guinea-pigs. *Biochem Pharmacol* 1979;28:3299–3305.
- Chamulitrat W, Burhenne J, Rehlen T, Pathil A, Stremmel W: Bile salt-phospholipid conjugate ursodeoxycholate lysophosphatidylethanolamide as a hepatoprotective agent. *Hepatology* 2009;50:143–154.
- Becker C, Fantini MC, Wirtz S, Nikolaev A, Kiesslich R, Lehr HA, et al: In vivo imaging of colitis and colon cancer development in mice using high resolution chromoendoscopy. *Gut* 2005;54:950–954.
- Engel MA, Kellermann CA, Rau T, Burnat G, Hahn EG, Konturek PC: Ulcerative colitis in AKR mice is attenuated by intraperitoneally administered anandamide. *J Physiol Pharmacol* 2008;59:673–689.
- Marshall W, Lapsley M, Day A, Ayling R: *Clinical Biochemistry: Metabolic and Clinical Aspects*, ed 3. London, Elsevier Health Science, 2014.
- Langhorst J, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ: Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. *Am J Gastroenterol* 2008;103:162–169.
- Stremmel W, Staffer S, Wannhoff A, Pathil A: The overall fatty acid absorption controlled by basolateral chylomicron excretion under regulation of p-JNK1. *Biochim Biophys Acta* 2017;1862:917–928.
- Stremmel W, Ehehalt R, Staffer S, Stoffels S, Mohr A, Karner M, et al: Mucosal protection by phosphatidylcholine. *Dig Dis* 2012;30(suppl 3):85–91.
- Wexler HM: Bacteroides: the good, the bad, and the nitty-gritty. *Clin Microbiol Rev* 2007;20:593–621.
- Karner M, Kocjan A, Stein J, Schreiber S, von Boyen G, Uebel P, 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;109:1041–1051.
- Stremmel W, Merle U, Zahn A, Autschbach F, Hinz U, Ehehalt R: Retarded release phosphatidylcholine benefits patients with chronic active ulcerative colitis. *Gut* 2005;54:966–971.
- Stremmel W, Ehehalt R, Autschbach F, Karner M: Phosphatidylcholine for steroid-refractory chronic ulcerative colitis: a randomized trial. *Ann Intern Med* 2007;147:603–610.
- Prantera C, Lochs H, Grimaldi M, Danese S, Scribano ML, Gionchetti P, et al: Rifaximin-extended intestinal release induces remission in patients with moderately active Crohn’s disease. *Gastroenterology* 2012;142:473–481.e4.
- Dianda L, Hanby AM, Wright NA, Sebesteny A, Hayday AC, Owen MJ: T cell receptor-alpha beta-deficient mice fail to develop colitis in the absence of a microbial environment. *Am J Pathol* 1997;150:91–97.
- Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, et al: Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect Immun* 1998;66:5224–5231.