



Low levels of bile acids increase bacterial uptake in colonic biopsies from patients with collagenous colitis in remission

Münch A*, Söderholm JD, Ost A, Carlsson AH, Magnusson KE and Ström M

¹Department of Clinical and Experimental Medicine, Division of Gastroenterology and Hepatology, Linköping University, Sweden

*Corresponding Author: Munch A, Department of Clinical and Experimental Medicine, Division of Gastroenterology and Hepatology, Linköping University, Sweden. Email id: andreas.munch@lio.se

Cite this article: Investigation in blood Leukocytes and Neutrophils in Periparturient Dairy Cow. Sci J of Gas and Hepa. 2018; 1(1): 001-006.

Submitted: 10 September 2018; **Approved:** 14 September 2018; **Published:** 15 September 2018

ABSTRACT

Background: Patients with collagenous colitis have an impaired mucosal barrier. Moreover, collagenous colitis is associated with bile acid malabsorption. Bile acids can increase bacterial mucosal uptake in humans. Mucosal barrier function was investigated by exposing colonic biopsies to chenodeoxycholic acid (CDCA) or deoxycholic acid (DCA) in Ussing chamber experiments.

Aim: To find if low levels of bile acids increase bacterial uptake in colonic biopsies from collagenous colitis patients.

METHODS: The study comprised 33 individuals; 25 with collagenous colitis (14 in clinical remission without treatment, 11 with active disease and 10 examined in clinical remission resulting from treatment with 6 mg budesonide); eight healthy individuals undergoing screening colonoscopy served as controls. Endoscopic biopsies from the sigmoid colon were mounted in modified Ussing chambers and assessed for short-circuit current (I_{sc}), potential difference, trans-epithelial resistance and transmucosal passage of Escherichia coli K12 after adding 100 µmol/L CDCA or DCA.

Results: When adding 100 µmol/L CDCA or DCA, bacterial uptake increased fourfold in biopsies of patients in remission; CDCA 6.5 units [2.5-9.8] and DCA 6.2 units [2.1-22] (median [IQR]), compared with uptake in biopsies without added bile acids 1.6 units [1.1-3] (P=0.004 and P=0.01 respectively). In active disease and in patients in remission due to budesonide treatment, bile acids did not affect bacterial uptake. Confocal microscopy revealed trans-epithelial passage of E. coli K12 within 30 min.

Conclusions: Low concentrations of dihydroxy-bile acids exacerbate mucosal barrier dysfunction in colonic biopsies of patients with collagenous colitis in remission. This allows a substantially increased bacterial uptake, which may contribute to recurrence of inflammation. **Keywords:** Dairy Cows; Periparturient Period; Leukocytes; Neutrophils; CD18; CD62L; Vitamin A; Vitamin E; Calcium; Phosphorous; Potassium; Sodium; Magnesium; Selenium; Copper; Zinc

INTRODUCTION

Collagenous Colitis (CC) is an inflammatory bowel disease (IBD) of unknown origin having an incidence rate of 5–6 / 100 000 inhabitants.^{1–3} It affects not only mainly elderly women (mean age 65) but also men and can occur at any age.¹ CC patients present clinically with frequent, nonbloody diarrhoea, abdominal pain and weight loss. The diagnosis can be established by histology only as colonic biopsies must show a thickened subepithelial collagenous layer and signs of inflammation in lamina propria. Many pathophysiological mechanisms have been suggested, such as autoimmunity [4,5] infection,⁶ drugs⁷ and bile acid malabsorption.⁸ Faecal stream diversion by surgery leads to histological remission in CC patients. When bowel continuity is restored, recurrence of mucosal inflammation often ensues, making it likely that an unknown luminal agent has triggered the inflammatory process [9].

Inflammatory bowel diseases, e.g. Crohn's disease and ulcerative colitis, are believed to have a leaky epithelial barrier, leading to increased uptake of proteins and antigens which induce an immunological response in the mucosa.¹⁰ In a previous study we showed that biopsies from patients with CC had a disturbed colonic barrier function even when they were in clinical remission.¹¹ Bile acids can stimulate secretion in the colon and in many animal studies chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA) in concentrations between 1 and 8 mmol / L have produced a dose-related increase in paracellular mucosal permeability and also damaged the mucosa (epitheliolysis), as verified by light and electron microscopy [12]. Recently we have also found that dihydroxy-bile acids in concentrations as low as 100 µmol / L of can increase mucosal bacterial uptake in human colonic biopsy tissue [13].

In view of these findings we speculate that even low concentrations of bile acids can further increase Escherichia coli uptake in colonic biopsies from CC patients, thus testing the hypothesis that bile acids may affect mucosal barrier function in CC. As budesonide has the best documented efficacy for treating CC, we also investigated the effect of bile acids on mucosal bacterial uptake in patients undergoing budesonide treatment.

METHOD

From December 2005 to February 2009, patients with known CC were asked to register their bowel movements over 1 week in a diary before undergoing sigmoidoscopy with biopsy sampling from the mid-part of the sigmoid colon. Mean stool frequency and the number of watery stools per day / week were calculated to categorise the patients as: in remission or with active disease (relapse), as scored ad modum Hjortswang et al.¹⁴ Active disease (relapse) was defined as producing a mean of ≥ 3 stools / day, of which a mean of at least one watery stool / day over a 1-week registration.

The results are based on 25 cases of CC (21 women and four men, mean age 66 years), 14 in clinical remission without treatment, 11 with active disease and 10 undergoing budesonide treatment. Of the 11 patients with active disease, 10 were treated with budesonide (Entocort, AstraZeneca, Sönderå lje, Sweden) for at least 6 weeks. At re-examination (sigmoidoscopy) all patients were in clinical remission, taking 6 mg budesonide daily. In all patients drug-induced CC was ruled out and in patients with active disease stool, cultures were negative. Two patients had concomitant coeliac disease but had normalised histology due to strict gluten-free diet. One patient in remission and two in the group 'active disease' had concomitant bile acid malabsorption measured with selenium-labelled homocholeic acid-aurine (SeHCAT) (cutoff <10%). None of these patients had had any benefit of bile acids resins and no patient received cholestyramine during the study.

Eight patients (six women and two men, mean age 62 years) who underwent colonoscopy for malignancy screening and who had normal histology were used as controls. Patients taking a NSAID or an immunosuppressive agent were excluded. All patients gave their informed consent and the study was approved by the Ethics Committee, Faculty of Health Sciences, Linköping, Sweden.

Endoscopy

Bowel preparation required 3–4 litres of polyethylene glycol (Laxabon, Bio-Phausia AB, Stockholm, Sweden). Sixteen biopsy samples were taken with biopsy forceps (CBF 2.5–230; Cook Sweden AB, Askim, Sweden) from the mid-part of the sigmoid colon: two for histology, 12 for the Ussing chamber studies [with the

following distribution: two controls, two for *E. coli*, two *E. coli* + CDCA, two *E. coli* + DCA and four for confocal microscopy (different time intervals)] and two to be held in reserve.

Using Chamber Experiments

The biopsies were taken in ice-cold oxygenated modified Krebs-Ringer bicarbonate buffer to the laboratory within 15 min. They were mounted in modified 1.5 mL Ussing chambers (Grass & Sweetana; Harvard Apparatus Inc., Holliston, MA, USA) with an exposed tissue area of 1.76 mm² using the technique previously described by Wallon et al.¹⁵ Oxygenation was continuous; pH was kept at 7.4 and temperature maintained at 37 °C. The mucosal compartment was filled with 1.5 mL 10 mM mannitol in Krebs buffer and the serosal compartment was filled with 1.5 mL 10 mM glucose in Krebs buffer. Experiments started with a 40-min equilibration period to establish a steady state. Trans-epithelial potential difference (PD), short-circuit current (I_{sc}) and trans-epithelial resistance (TER) were studied for 120 min in all specimens, using one pair of Ag / AgCl electrodes with agar-salt bridges and one pair of current-giving platinum electrodes. Only three biopsies had to be excluded because of an initial PD greater than 0.5 mV which implies uncertain viability or leakage,¹⁵ but all other biopsies had a stable PD value (<0.5 mV) for the duration of the experiment (data not shown). Passage of chemically killed *E. coli* K-12

All biopsies were investigated for uptake of chemically killed, fluorescein-conjugated *E. coli* K-12 bioparticles (Molecular Probes, Leiden, the Netherlands). These bacteria are killed with paraformaldehyde, which stops their reproduction but retains antigenicity. A concentration corresponding to 1.0 • 10⁸ CFU / mL was added to the mucosal compartment as previously described by Keita et al.¹⁶ After 2 h the entire content of the serosal compartment was analysed at 488 nm in a fluorimeter (Cary Eclipse, Varian, California, CA, USA) where 1 unit corresponds to 3 • 10³ CFU / mL.

Mucosal exposure to bile acids

Sodium-chenodeoxycholate (3a, 7a-dihydroxyl-5b-cholan-24-oic acid, >97%; Sigma, St Louis, MO, USA) and sodium-deoxycholic acid (3a, 12a-dihydroxyl-5b-cholan-24-oic acid, SigmaUltra >99%) were diluted with mannitol Krebs to obtain concentrations of 100 μmol / L. We chose this concentration as it reflects levels present in the colon and showed effects on barrier function in our previous study.¹³ After a 40-min equilibration, mannitol Krebs containing CDCA or DCA was added to the mucosal compartment of two biopsy samples.

HISTOLOGY

All biopsies were examined by one and the same pathologist (ÅO, blinded); two from the sigmoid colon were taken at each investigation and were stained with haematoxylin-eosin and van Gieson. The degree of surface epithelial cell degeneration was assessed in arbitrary units (0 = none, 1 = mild, 2 = moderate, 3 = severe). The thickness of the collagenous band was measured in five different areas and the mean value was determined.

Immunohistochemical staining for CD3 was also performed according to routine procedures. The number of intra-epithelial lymphocytes (IEL) / 100 enterocytes (mean value of three counts) was determined. The infiltration of mononuclear cells (lymphocytes and plasma cells) into the lamina propria was defined in arbitrary units (0 = none, 1 = mild, 2 = moderate, 3 = severe).

Confocal Microscopy Studies

For studies on bacterial transport in colonic tissue, samples were fixed in 4% formalin after 30 and 60 min and mounted in Cryo Mount, Cryosections (Sacura, CA, USA) (10 μm). They were cut using a microtome (Leica, Wetzlar, Germany) and dried overnight at room temperature on polysine glass slides. After cooling to room temperature, the sections were rinsed three times in PBS, and then blocked in PBS containing 5% BSA. Prolong Gold with DAPI was used as mounting medium to achieve concurrent nuclear and chromosome staining. Cryosections were examined using confocal laser scanning microscopy (BioRad Radiance 2000 MP with LaserSharp 2000 software, Carl Zeiss AB, Oberkochen, Germany).

Statistics

Data are presented as median and 25–75th percentiles. Comparisons between the groups were initially done with Kruskal–Wallis and further analysed with Mann–Whitney tests. To compare patients before vs. after budesonide treatment, the Wilcoxon's matched-pairs signed-rank test was used. A two-sided P-value <0.05 was considered significant.

RESULTS

Histology

The histology in patients in clinical remission and findings before and after short-term budesonide treatment in patients with active CC are reported in Table 1. No significant change was evident before vs. after budesonide treatment. The inflammation in lamina propria was significantly milder in patients in remission than in patients in remission due to budesonide treatment (1 [0–2] vs. 2 [1–2] [IQR]; P < 0.01).

Escherichia coli passage

As shown in our previous study,¹¹ uptake of *E. coli* through the colonic epithelium was increased in all CC groups compared with controls (P = 0.05). By adding 100 μmol / L of CDCA or DCA to biopsies from patients with CC in remission, the already increased bacterial uptake compared with controls was further augmented by factor 4 (Figure 1). In patients with active disease, no further increase in bacterial passage was induced by bile acids, as was also the case when the individuals were receiving budesonide treatment.

There were no significant differences between the groups of patients with CC when bile acids were added.

Electrophysiological Measurements (TER, I_{sc}, PD)

The increased total wall resistance (TER) at the beginning of the study in CC patients with active disease compared with controls [47 (38–53) vs. 34 (27–37) Ohm cm², P < 0.05] is

Table 1 : Histology in patients with clinical remission and findings before and after 6 weeks budesonide treatment in patients with active collagenous colitis

Active disease before budesonide	Remission after 6 weeks on budesonide	Remission without budesonide	P-value
Histology (n = 11)	(n = 10)	(n = 14)	P-value
Degeneration	1 (0–3)*	0 (0–3)	N.S.
Collagenous band (Im)	15 (4–55)*	17 (7–25)	N.S.
IEL	30 (4–52)*	27 (6–49)	N.S.
Lamina propria IEL, intra-epithelial lymphocytes	2 (0–3)*	2 (1–2)	N.S.
		1 (0–2)*	<0.05

The groups 'remission without budesonide' and 'active disease' differed (*P < 0.05) but there were no significant changes before vs. after 6 weeks budesonide treatment. The inflammation in lamina propria was significantly more severe in patients in remission with budesonide treatment than in patients in remission without budesonide (P < 0.01). The data are presented as median a consequence of the thickened subepithelial collagenous layer and an inflammatory cell infiltration leading to submucosal oedema, as discussed in our earlier study.¹¹ Otherwise there were no significant differences in TER between the groups at baseline (0 min). Addition of 100 μmol / L CDCA or DCA to biopsies from patients in clinical remission, with active disease, or receiving budesonide treatment did not cause any significant changes (delta values) in TER during the 120 min experiment period. This indicates that the tight junctions were not affected by this concentration.

Concerning the short-circuit current (I_{sc}), no significant changes were seen in any of the groups when 100 μmol / L CDCA or DCA was added and the PD in all biopsies was stable during the 120 min experiment (data not shown).

Confocal Microscopy

Figure 2 shows that *E. coli* K12 crossed the epithelium within 30 min of adding bile acids and was found on the basolateral side of the epithelial cells. Imaging of the trans-epithelial passage of *E.*

coli did not reveal which route (para- / transcellular) was predominant.

DISCUSSION

We demonstrated recently that CC is associated with increased transmural passage of *E. coli* K12 bacteria and

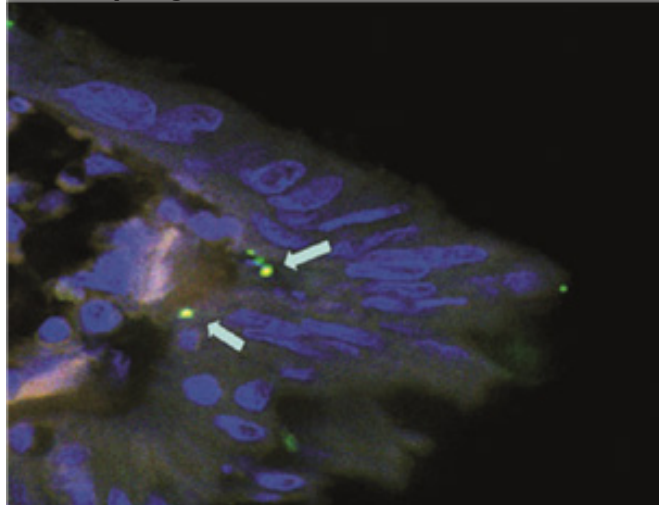


Figure 2: Visualisation of *E. coli* K12 on the basolateral side of the human intestinal epithelium (arrow). White = Phalloidin, F-actin; green = *E. coli* K12; blue = DAPI nuclear stain ($\times 63$ objective, $\times 2$ zoom).

- that the impaired barrier function is present in biopsies from patients in clinical remission as well as with active disease.¹¹ As up to 44% of CC patients have an accommodating bile acid malabsorption,⁸ we wanted to test the hypothesis that bile acids may influence barrier function in CC. The novel findings in the present study were that dihydroxy-bile acids, in concentrations normally found in the colon, exacerbated the already impaired mucosal barrier function by increasing bacterial uptake fourfold in patients in clinical remission. In contrast, biopsies from patients with active disease or in clinical remission following budesonide treatment were not affected by bile acids and no increased bacterial uptake was evident. Speculatively, this vulnerability to bile acids may offer an explanation for the rapid clinical recurrence often seen after withdrawing budesonide treatment of CC.

Hamilton et al.¹⁷ reported that the total caecal concentration of 3-hydroxy bile acids in humans was 600–300 $\mu\text{mol/L}$. As CDCA constitutes 7–8% (mean \pm s.d.) and DCA 34–16% of the total bile acid composition, a concentration of 100 $\mu\text{mol/L}$ CDCA and DCA lies within the physiological range in the caecum. Furthermore, stable epithelial PD values during our experiments contradicted cytotoxicity. In these concentrations, bile acids do not act as detergents,¹⁸ but have more specific, receptor-mediated effects. Bile acids can interact with the intestinal mucosa in many ways and it has been demonstrated that they modulate tight junction structures and barrier function in Caco-2 monolayers by activating the epithelial growth factor receptor¹⁹ or generating reactive oxygen species.²⁰ Nonpathogenic *E. coli* strains have been shown to ameliorate epithelial barrier function and mediate tight junction protein rearrangement.^{21, 22} In an earlier study,¹³ we showed that the combination of *E. coli* K12 and 100 $\mu\text{mol/L}$ dihydroxy bile acids enhanced bacterial passage and reduced TER in colonic biopsies from healthy subjects, which suggested effects on tight junctions. Moreover, confocal microscopy revealed that the bacteria passed the mucosa within 30 min.

Mucosal barrier dysfunction is believed to play a major role in the pathogenesis of IBD,²³ but to our knowledge no study has been conducted to consider the possible action that bile acids might have on mucosal integrity in humans affected by IBD. Interestingly, our study suggests that the already impaired barrier in active disease is not exacerbated by adding bile acid. This raises

the question as to which structures or mechanisms are involved in biopsies from patients in clinical remission that make them more vulnerable or hyperreactive to bile acids. Similar observations were made in our previous study on ileal biopsies from Crohn's patients, where stimulation with sodium caprate, a tight junction modulator, induced a more pronounced increase in flux of [51].

Cr-EDTA, a paracellular marker, and reduced TER more in non-inflamed than in inflamed tissue.²⁴

Faecal bile acid concentrations in CC patients have not been analysed previously, but a pathological SeHCAT in 44% of CC patients⁸ does reflect higher bile acid loss via the colon. CC presents with increased numbers of IEL also in the terminal ileum,²⁵ indirectly suggesting functional alterations. It can therefore be speculated that bile acid reabsorption via the active sodium bile acid transporter is affected. Moreover, increased intestinal motility in diarrhoeal disorders can per se lead to higher 'spillover' of primary bile acids in the right colon. Holmquist et al.²⁶ reported that patients with ulcerative colitis who had bile acid malabsorption showed a high degree of mucosal inflammation in the right colon at colonoscopy. The right colon is also the main site of the classical histological findings of CC.²⁷ Furthermore Ejderhamn et al.²⁸ found that children with IBD in clinical remission had significantly increased bile acid levels in faeces and faecal water. In Crohn's disease, faecal bile acids are also increased with an altered bile acid pattern showing a predominance of primary bile acids.²⁹ Considering these observations together, it could be speculated that luminal bile acids, in susceptible hosts, could be promoters of colonic inflammation. The possible pathogenic role of bile acids in CC is further corroborated by the symptomatic effects of bile acid-binding resins, such as cholestyramine, that are clinically effective and ameliorate diarrhoeal symptoms in CC.⁸

With consideration that bile acid concentrations and intestinal inflammation are higher in the right colon, it would have been favourable to study biopsies of the ascending colon. This was difficult because of practical reasons and some patients would have refused repeated colonoscopies. On the other hand, we have not found any correlation between the degree of histological manifestations and bacterial uptake in our prior study.¹¹

Budesonide has a well-documented efficacy for inducing and maintaining remission in CC.³⁰ Steroids have a well-known immunosuppressive effect and they bind to the glucocorticoid complex suppressing myosin light chain kinase (MLCK) activity which mediates intestinal tight junction permeability.³¹ Boivin et al. demonstrated that glucocorticoids can inhibit a TNF- α -induced TJ barrier defect. Furthermore, budesonide increases ileal bile acid transport in CC and thereby decreases bile acid load on the colon as shown by Bajor et al.³² These findings lead to the speculation that the good clinical efficacy of budesonide might also partially rely on modulatory effects on bile acid induced diarrhoea or mucosal damage in CC.

In the present study on the other hand, we found no effect of low concentrations of bile acids on biopsies from patients with budesonide treatment. As these biopsies had a higher background level of inflammation and bacterial uptake, this might have overshadowed the experimental deleterious effect of bile acids.

CC runs a mainly chronic, intermittent course. It is still not known what causes the intestinal inflammation or triggers a recurrence. Our data suggest that asymptomatic patients have an underlying barrier defect and increased mucosal vulnerability to bile acids, leading to increased bacterial uptake through the colonic epithelium. These detrimental effects on mucosal barrier function could facilitate initiation and perpetuation of mucosal inflammation in CC. Our findings may also offer an explanation for the rapid clinical relapse that is often seen after ending the immunosuppressive and barrier protecting therapy with budesonide.³³

ACKNOWLEDGEMENTS

Declaration of personal interests: None. Declaration of funding interests: This study was funded by Swedish Research Council – Medicine (JDS) and the National funding for medical training and research (grant number LIO-10576).

REFERENCES

- Olesen M, Eriksson S, Bohr J, et al. Microscopic colitis: a common diarrhoeal disease. An epidemiologic study in Örebro, Sweden, 1993–1998. *Gut* 2004; 53: 346–50.
- Wickbom A, Nylin N, Eriksson S, et al. Stable incidence of collagenous colitis and lymphocytic colitis in Örebro, Sweden 1999–2008. *Gut* 2009; 58(Suppl. II): A58.
- Pardi DS, Loftus EV, Smyrk TC, et al. The epidemiology of microscopic colitis: a population based study in Olmsted County, Minnesota. *Gut* 2007; 56: 504–8.
- Bohr J, Tysk C, Yang P, et al. Autoantibodies and immunoglobulins in collagenous colitis. *Am J Gastroenterol* 1992; 87: 780–3.
- Freeman HJ. Perinuclear autoneutrophil cytoplasmic antibodies in collagenous or lymphocytic colitis with or without celiac disease. *Can J Gastroenterol* 1997; 11: 417–20.
- Bohr J, Nordfelth R, Järnerot G, et al. Yersinia species in collagenous colitis: a serologic study. *Scand J Gastroenterol* 2002; 37: 711–7.
- Beaugerie L, Pardi DS. Review article: drug-induced microscopic colitis – proposal for a scoring system and review of the literature. *Aliment Pharmacol Ther* 2005; 22: 277–84.
- Ung KA, Gillberg R, Kilander A, et al. Role of bile acids and bile acid binding agents in patients with collagenous colitis. *Gut* 2000; 46: 170–5.
- Järnerot G, Tysk C, Bohr J, Eriksson S. Collagenous colitis and fecal stream diversion. *Gastroenterology* 1995; 109: 449–55.
- Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet* 2007; 369: 1627–40.
- Münch A, Söderholm JD, Ost A, Ström M. Increased transmucosal uptake of E. coli K12 in collagenous colitis persists after budesonide treatment. *Am J Gastroenterol* 2009; 104: 679–85.
- Chadwick VS, Gainella TS, Carlson GL, et al. Effects of molecular structure on bile acid-induced alterations in absorptive function, permeability and morphology in the perfused rabbit colon. *J Lab Clin Med* 1979; 94: 661–74.
- Münch A, Ström M, Söderholm JD. Dihydroxy bile acids increase mucosal permeability and bacterial uptake in human colon biopsies. *Scand J Gastroenterol* 2007; 42: 1–8.
- Hjortswang H, Tysk C, Bohr J, et al. Defining clinical criteria for clinical remission and disease activity in collagenous colitis. *Inflamm Bowel Dis* 2009; 15: 1875–81.
- Wallon C, Braaf Y, Wolving M, et al. Endoscopic biopsies in Ussing chamber evaluated for studies of macromolecular permeability in the human colon. *Scand J Gastroenterol* 2005; 40: 586–95.
- Keita AV, Gullberg E, Ericson A, et al. Characterization of antigen and bacterial transport in the follicle-associated epithelium of human ileum. *Lab Invest* 2006; 86: 504–16.
- Hamilton JP, Xie G, Raufman JP, et al. Human cecal bile acids: concentration and spectrum. *Am J Physiol Gastrointest Liver Physiol* 2007; 293: 256–63.
- Rafter JJ, Branting C. Bile acids – interaction with the intestinal mucosa. *Eur J Cancer Prev* 1991; 1(Suppl. 2): 49–54.
- Raimondi FR, Santoro P, Barone MV, et al. Bile acids modulate tight junction structure and barrier function of Caco-2 monolayer via EGFR activation. *Am J Physiol Gastrointest Liver Physiol* 2008; 294: 906–13.
- Araki Y, Katoh T, Ogawa A, et al. Bile acids modulate transepithelial permeability via the generation of reactive oxygen species in the Caco-2 cell line. *Free Radic Biol Med* 2005; 39: 769–80.
- Zareie M, Riff J, Donato K, et al. Novel effects of the prototype translocating Escherichia coli, strain C25 on intestinal epithelial structure and barrier function. *Cell Microbiol* 2005; 7: 1782–97.
- Otte JM, Podolsky DK. Functional modulation of enterocytes by Gram-positive and Gram-negative microorganism. *Am J Physiol Gastrointest Liver Physiol* 2004; 286: 613–26.
- Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007; 448: 427–34.
- Söderholm JD, Olaison G, Peterson KH, et al. Augmented tight junction permeability increase in non-inflamed ileal mucosa in Crohn's disease in response to sodium caprate in Ussing chambers – a distribution of perijunctional actin regulation? *Gut* 2002; 50: 307–13.
- Sapp H, Ithamukkala S, Brien TP, et al. The terminal ileum is affected in patients with lymphocytic and collagenous colitis. *Am J Surg Pathol* 2002; 26: 1484–92.
- Holmquist L, Andersson H, Rudic N, et al. Bile acids malabsorption in children and adolescents with chronic colitis. *Scand J Gastroenterol* 1986; 21: 87–92.
- Tanaka M, Mazzoleni G, Riddell RH. Distribution of collagenous colitis: utility of flexible sigmoidoscopy. *Gut* 1992; 33: 65–70.
- Ejderhamn J, Rafter JJ, Strandvik B. Faecal bile acid excretion in children with inflammatory bowel disease. *Gut* 1991; 32: 1346–51.
- Kruis W, Kalek HD, Stellaard F, et al. Altered fecal bile acid pattern in patients with inflammatory bowel disease. *Digestion* 1986; 35: 189–98.
- Chande N, MacDonald JK, McDonald JWD. Interventions for treating microscopic colitis: a Cochrane inflammatory bowel disease and functional bowel disorders review group systemic review of randomized trials. *Am J Gastroenterol* 2009; 104: 235–41.
- Boivin MA, Ye D, Kennedy JC, et al. Mechanism of glucocorticoid regulation of the intestinal tight junction barrier. *Am J Physiol Gastrointest Liver Physiol* 2007; 292: 590–8.
- Bajor A, Kilander A, Galman C, Rudling M, Ung KA. Budesonide treatment is associated with increased bile acid absorption in collagenous colitis. *Aliment Pharmacol Ther* 2006; 24: 1643–9.
- Miehlke S, Madisch A, Voss C, et al. Long-term follow-up of collagenous colitis after induction of clinical remission with budesonide. *Aliment Pharmacol Ther* 2005; 22: 1115–9.