Intestinal secretory and absorptive function in *Trichinella spiralis* mouse model of postinfective gut dysfunction: role of bile acids

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ABSTRACT

Objective: Observations showing that bile acid malabsorption is frequent in irritable bowel syndrome (IBS) suggest that alterations in bile acid-induced secretion and absorption could contribute to IBS-associated diarrhoea. The secretory response to bile acids, fluid transport and bile absorption was examined in intestinal tissues from a *Trichinella spiralis* mouse model of postinfectious gut dysfunction *in vitro*. Changes in the protein expression of apical sodium-dependent bile acid transporter (ASBT) were also measured.

Design: *T. spiralis*-infected mice were killed at 18 and 25 days postinfection. Jejunal, ileal, proximal and distal colon segments were exposed to taurodeoxycholic acid (TDCA) or cholic acid. Short circuit current (SCC) increases were determined. Tritiated taurocholic acid (3H-TCA) absorption was determined in everted jejunal and ileal sacs. ASBT protein expression was determined by Western blot analysis and immunohistochemistry.

Results: Basal SCC increased in ileum and distal colon at 18 and 25 days postinfection, respectively. Ileal SCC responses to TDCA and cholic acid were enhanced at 18 days postinfection. Distal colon SCC response to TDCA was raised at 18 days postinfection but was significantly reduced by 25 days. Ileal 3H-TCA uptake was significantly reduced at 18 and 25 days postinfection. Surprisingly, increased ASBT expression was observed in infected animals.

Conclusions: In a *T. spiralis* model of postinfectious gut dysfunction, decreased bile absorption and enhanced secretion in response to bile acids was observed. Decreased absorption was not, however, caused by decreased ASBT as increased expression was observed. If similar events occur postinfection, the combined effects of these disturbances may contribute to some symptoms observed in postinfectious IBS patients.

Irritable bowel syndrome (IBS) is an extremely common disorder that affects up to 20% of the general population and is responsible for almost half of the referrals to gastroenterologists.[1 2] Surprisingly, the cause of IBS is poorly understood and several pathophysiological mechanisms have been implicated.[3–5] Recent clinical investigations have demonstrated that approximately 25% of patients with IBS have a history of infection.[6–8] This condition, named postinfectious irritable bowel syndrome (PI-IBS), may be induced in humans by bacterial (*Campylobacter, Salmonella*) or in mice by parasitic (*Trichinella spiralis*) agents. In both clinical and animal studies of PI-IBS, altered gut physiology has been described, which persists even after the infection and associated inflammation resolves.[8–10] Persistent chronic diarrhoea is also a common problem associated with IBS and is more frequently associated with PI-IBS.[11] Although bile acid malabsorption (BAM) has generally been regarded an infrequent cause of chronic diarrhoea, recent improvements in the techniques employed for assessing BAM have demonstrated that it is a much more common cause of diarrhoea than originally considered.[12 13] This has been highlighted by the recent and unexpected evidence that BAM was observed in 33% of patients with diarrhoea-predominant IBS.[13]

Bile acids are synthesised in the liver and secreted into the small intestine where they facilitate fat and fat-soluble vitamin absorption. Although some bile uptake occurs in the jejunum, the main route for circulation of bile back into the liver is by active reabsorption in the terminal ileum by the apical sodium-dependent bile acid transporter (ASBT). Dysfunction of ASBT is accompanied by an interruption in the enterohepatic bile circulation, allowing bile acids to enter the colon in increased concentrations.[14 15] This subsequently induces diarrhoea as bile acids stimulate chloride ion (Cl^-) secretion and powerful propagating contractions in the colon.[16] Although IBS has generally been considered a motility disorder, it seems likely that the condition may involve changes in fluid and electrolyte transport across the intestinal epithelium, because diarrhoea and mucus hypersecretion are well-recognised features. In addition, intestinal secretory mechanisms may be more sensitive to secretogogues, such as bile acids, during IBS. Oddsson and colleagues[17] have shown that the small intestine in IBS patients has a greater secretory response to low bile acid concentrations.

Recent work in our laboratory and by others has established the characteristics of bile acid-induced secretion and ileal bile acid absorption in normal and mast cell-deficient mice.[18–21] Similar studies have not been performed in a murine model of postinfectious gut dysfunction. The *T. spiralis*-infected mouse is a widely acknowledged model of postinfectious gut dysfunction in which visceral hypersensitivity and persistently altered motility, which mimic the hyperreactive state in IBS, are observed.[10 22] *T. spiralis* infection has two phases. Postinfectious gut dysfunction occurs after the enteric phase, when the worm is expelled from the intestine. There is also a skeletal muscle phase during which the worm is present in muscle (for the duration of the mouse's life) despite gut expulsion. Although infection with the nematode initially generates an intestinal inflammatory

response that resolves after worm expulsion from the intestine, functional changes such as increased motility, visceral hypersensitivitity and increased muscle thickness persist. The current study determined the secretory effects of cholic acid and taurodeoxycholic acid (TDCA) in the small intestine and colon of *T. spiralis*-infected mice at two postinfective timepoints. In addition, studies were performed to investigate whether passive bile uptake in the jejunum and active bile absorption in the terminal ileum was also impaired in these mice. Furthermore, changes in the expression of ASBT after infection were determined in an attempt to correlate these with any observed differences in bile salt absorption.

MATERIALS AND METHODS

Animals

Experiments were performed on intestinal tissues from *T. spiralis*-infected and non-infected mice killed by cervical dislocation in accordance with UK Home Office regulations and with local Ethical Committee approval. Male Swiss mice (age 12–13 weeks) were obtained from Sheffield Field Laboratories and were allowed free access to food and water.

Infection with *T. spiralis*

Stock mice infected with *T. spiralis* were killed to obtain larvae for infecting mice to be used in experimental procedures. Larvae were recovered from stock mice by pepsin (0.5%) and hydrochloric acid (0.5%) digestion of the skeletal muscle as described by Castro and Fairbain.[23] Experimental male Swiss mice (25–30 g; Harlan International, Bicester, Oxon, UK) were orally infected by administering 300 larvae in a 0.2 ml saline suspension. This number of larvae produced a robust inflammatory response before worm expulsion at days 12–14 postinfection. Animals were killed at days 18 and 25 postinfection by cervical dislocation. Two timepoints allowed this study to determine whether changes in secretory responses to bile were maintained after recovery from the inflammatory insult. To confirm that experimental mice were indeed infected, a sample of the diaphragm was examined under the microscope and worms were identified within the muscle. A control group of sham mice were gavaged with saline in a similar manner and killed after 18–20 days.

Measurement of transintestinal electrical activity

Ussing chambers were used to measure changes in ion transport through the electrical correlate, short circuit current (SCC). Segments of jejunum (immediately distal to the ligament of Treitz), terminal ileum (6 cm before the caecum), proximal colon and distal colon were stripped of the outer muscle layers, which removed the myenteric plexus as well as the muscle coat but left intact the submucosal and mucosal plexus.[24] Each sheet was mounted in an Ussing chamber (0.5 cm^2 aperture) and incubated at 37°C in normal Krebs bicarbonate saline gassed with 95% oxygen–5% carbon dioxide. Normal Krebs contained (in mm): Na^+, 143.4; K^+, 5.9; Ca^+, 2.5; Mg^{2+}, 1.2; Cl^-, 125.7; HCO_3^-, 24.9; $H_2PO_4^-$, 1.2, SO_4^{2-}, 1.2. The serosal fluid contained 10 mmol glucose and the mucosal fluid 10 mml mannitol, and each had a volume of 5 ml. The potential difference was measured using salt bridge electrodes connected via calomel half cells to a differential input electrometer. Current was applied across the tissue via conductive plastic electrodes, and tissue resistance was determined from the potential difference change induced by a 50 μA current pulse, taking into account fluid resistance. The SCC generated by the sheets was calculated from the potential difference and resistance measurement using Ohm's law.

Tissue was allowed to stabilise for 15 minutes after mounting, and readings of electrical activity were subsequently taken at one minute intervals. After five minutes of basal readings, either cholic acid (Sigma, St Louis, Missouri, USA) or TDCA (Sigma) was added to the serosal side and readings were taken for a further 15 minutes. We have previously looked at the effects of both the mucosal and serosal application of several bile acids to the small intestine and found a concentration of 1 mmol to be effective only from the serosal side.[19] Therefore bile acid secretion is initiated by action at the serosal side of the enterocyte. The actual effective concentration is likely to be considerably less because of the diffusion barrier, represented by subepithelial tissues, which needs to be overcome. Furthermore, in the ileum, sodium-dependent bile acid absorption also increases SCC, so to avoid this component of the overall SCC change that occurs when bile acids are applied mucosally, serosal application (when absorption is not activated) was chosen. An aliquot of 100 μl cholic acid or TDCA, dissolved in ethanol and saline, respectively, was added to the 5 ml bathing solution to yield a final concentration of 1 mmol for both substances. Preliminary studies identified that neither ethanol nor saline, added serosally, had any significant effect on basal electrical activity. The SCC generated by the sheets after bile acid administration was calculated as described above using Ohm's law.

Measurement of intestinal fluid transport

The transport of fluid by the mucosa was measured in everted sacs taken from proximal jejunum and terminal ileum. A 5–7 cm intestinal segment, everted on a glass rod, was filled with 0.2 ml Krebs bicarbonate saline containing 10 mmol glucose (serosal fluid) and was incubated for 30 minutes in 15 ml Krebs bicarbonate saline containing 10 mmol mannitol (mucosal fluid) at 37°C in a shaking water bath. Results are expressed as mucosal fluid transport (MFT), which is the sum of the increase in the volume (weight of tissue) of serosal fluid in the sac after incubation (serosal fluid transport) and that taken up by the gut itself (gut fluid uptake) and values were related to the initial wet weight of the empty sac (ml/g initial wet weight/30 minutes).

Measurement of 3H-TCA absorption

The absorption of taurocholic acid (TCA) was also assessed in the same everted sacs by adding TCA (1 mmol; Sigma) together with 3H-TCA (2.5 μCi/100 ml; PerkinElmer Life Sciences, Boston, Massachusetts, USA) to the mucosal fluid. At the end of the incubation period the serosal fluid was collected. The sac was deproteinised using 10% sodium tungstate (1.25 ml) and 0.33 MH_2SO_4 (1.25 ml), homogenised and then filtered. Scintillation fluid (3 ml; Emulsifer-safe; Packard Biosciences, USA) was added to 100 μl samples of initial mucosal fluid, final mucosal fluid, final serosal fluid and gut homogenate, and radioactivity was determined using a liquid scintillation analyser (Packard TRI-CARB, 1900XR; Packard Biosciences, Pangbourne, Berkshire, UK). TCA absorption was expressed in two ways: first, as the amount taken up by the sac (μmol/g initial wet weight/30 minutes) and second as the T/M ratio, i.e. the ratio of the TCA concentration in the tissue water compared with its concentration in the mucosal fluid at the end of the incubation period, whereby a T/M ratio greater than 1 indicated active transport.

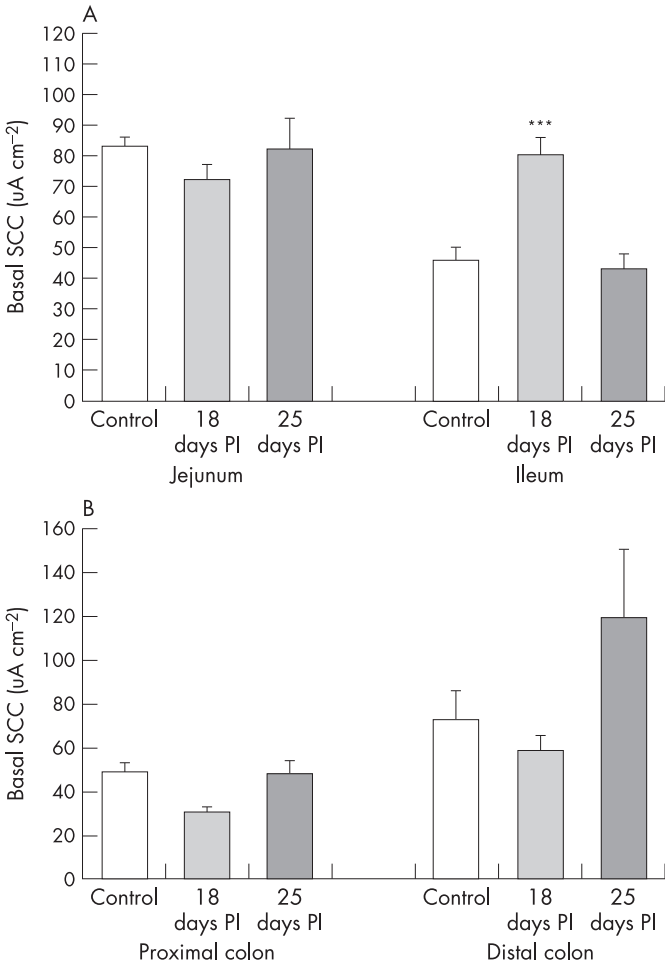

Figure 1 Postinfection (PI) changes in basal short circuit current (SCC) in the (A) small intestine and (B) colon of non-infected control and *Trichinella spiralis*-infected animals. Basal SCC was determined by applying a current across the stripped tissue before exposure to cholic acid or taurodeoxycholic acid (TDCA) in non-infected control and *T. spiralis*-infected animals. Results are expressed as mean values ± SEM. ***$p<0.001$ with $n = 6$ for each tissue sample.

Preparation of epithelial cell homogenates for ASBT expression studies

Epithelial cell homogenates were prepared from the three contiguous 3 cm segments of the most distal part of the small intestine. All further steps were performed with the preparations kept on ice. Segments were opened in the longitudinal axis and washed in isotonic saline (0.9% NaCl) to discard adhering luminal content. Mucosa were scraped with a clean glass rod. The mucosal scrapings of three animals were suspended in 5 ml buffer A (10 mmol Tris/HCl/0.13 mol NaCl/5 mmol EDTA, pH 7.4) and stirred gently for 30 minutes at 4°C. Cells were collected by centrifugation (3 min, 2000 rpm, 4°C) and suspended in 500 µl buffer B (10 mmol Tris/HCl/0.3 mol mannitol, pH 7.2) and 20 µl of a protease inhibitor cocktail (Roche, Hertfordshire, UK) to a concentration of approximately 1–2 µg/µl. The samples containing proteins were stored at −20°C until use. The protein concentration was determined by the Bradford method (Biorad Laboratories, Munchen, Germany). Samples were solubilised in 5× sodium dodecylsulfate (SDS) sample buffer containing 0.125 mmol Tris·Cl, pH 6.8, 10% SDS, 50% glycerol, 10% mercaptoethanol and 0.005% bromophenol blue, and then boiled at 100°C for 5 minutes.

Western blot analysis

Samples of control and 18 days postinfection animals were used. Each sample contained a pool of the enterocytes of the distal ileum of three animals. Equal amounts of protein (10 µg/lane) were loaded and separated by electrophoresis on 10% SDS–polyacrylamide gel. After electrophoresis, the separated proteins were transferred to a polyvinylidene difluoride transfer membrane (Immobilon, Millipore, Bedford, Massachusetts, USA). The membranes were blocked with 5% non-fat dry milk in 0.2% Tween 20/Tris-buffered saline for 2 hours at room temperature. A goat polyclonal antibody against ASBT 1 : 100 (Santa Cruz Biotechnology, Santa Cruz, California, USA) or a rabbit polyclonal antibody against β-actin 1 : 1000 (ABCAM, Cambridge, UK) were applied overnight at 4°C. After washing, a peroxidase-conjugated anti-goat or anti-rabbit 1 : 2000 (Dako, Glostrup, Denmark) antibody was added to the membranes and incubated for 2 hours at room temperature. Visualisation was achieved by an enhanced chemiluminiscence technique (Amersham Biosciences, Little Chalfont, Bucks, UK) on Kodak Biomax Light films. Protein bands were analysed by imaging densitometry. In control experiments, jejunum was used as a negative control.

Immunohistochemistry

Mouse ileal tissue (excised 3 cm proximal to the caecum) was fixed in 4% paraformaldehyde for two hours at room temperature. The tissue was mounted in OCT compound (BDH, Poole, Dorset, UK), frozen in dry ice–ethanol for 30 minutes and stored at −80°C. Sections (6 µm) of mouse ileum were stored at −80°C. Sections were air-dried for 30 minutes and washed in phosphate-buffered saline (PBS). After blocking with 30% normal donkey serum for four hours at room temperature, sections were incubated with a goat polyclonal antibody against ASBT 1 : 50 (Santa Cruz Biotechnology) overnight at 4°C. After washing with PBS, sections were incubated with a donkey anti-goat IgG-FITC 1 : 100 (Santa Cruz Biotechnology) for two hours at room temperature. After washing with PBS, coverslips were mounted with Vectashield mounting media (Vector Laboratories, Burlingame, California, USA) for visualisation of the fluorescein label at a magnification of ×40. In control experiments, the primary antibody was omitted.

Statistics and expression of results

Results are expressed as mean values ±1 SEM of the number of observations indicated. Data for small bowel and colon were analysed separately using two-way analysis of variance followed by Bonferroni post-test as appropriate; $p<0.05$ was considered significant.

RESULTS

Effects of cholic acid and TDCA on ion transport in the small intestine

Basal SCC

In the jejunum, basal SCC was unchanged at 18 or 25 days postinfection when compared with non-infected jejunal controls. In the ileum, however, basal SCC was significantly increased ($p<0.001$) at 18 but not 25 days postinfection when compared with non-infected ileal controls (fig 1A).

Response to bile acids

In the jejunum, the SCC response to both cholic acid (fig 2A) and TDCA (fig 2B) was unchanged at day 18 but reduced at day

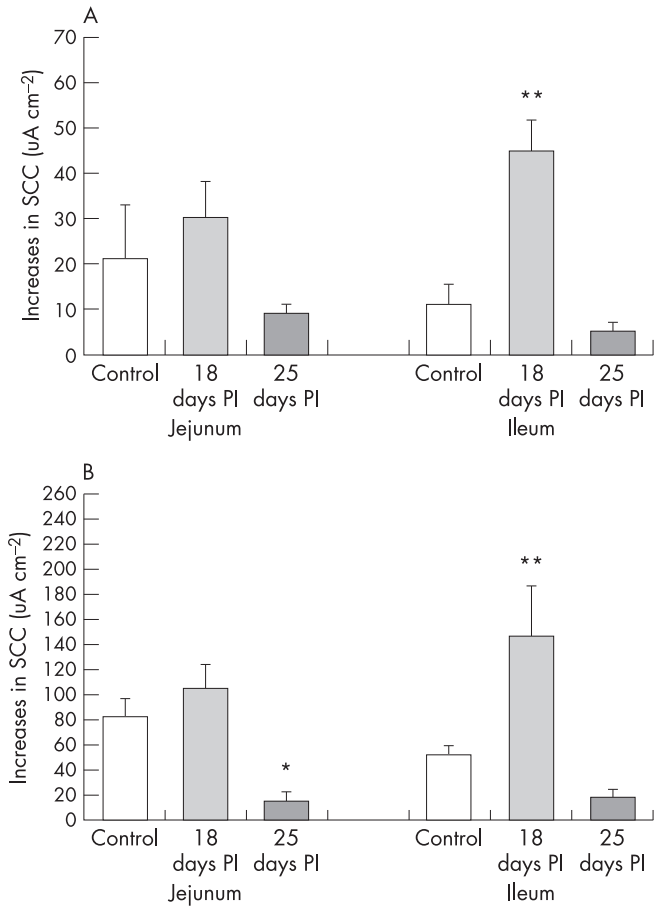

Figure 2 Effects of (A) cholic acid (1 mmol serosally) and (B) taurodeoxycholic acid (TDCA; 1 mmol serosally) on short circuit current (SCC) of stripped jejunum and ileum of non-infected control and *Trichinella spiralis*-infected animals. Results are expressed as mean values ± SEM. *p<0.05, **p<0.01 with n = 6 for each tissue sample. PI, Postinfection.

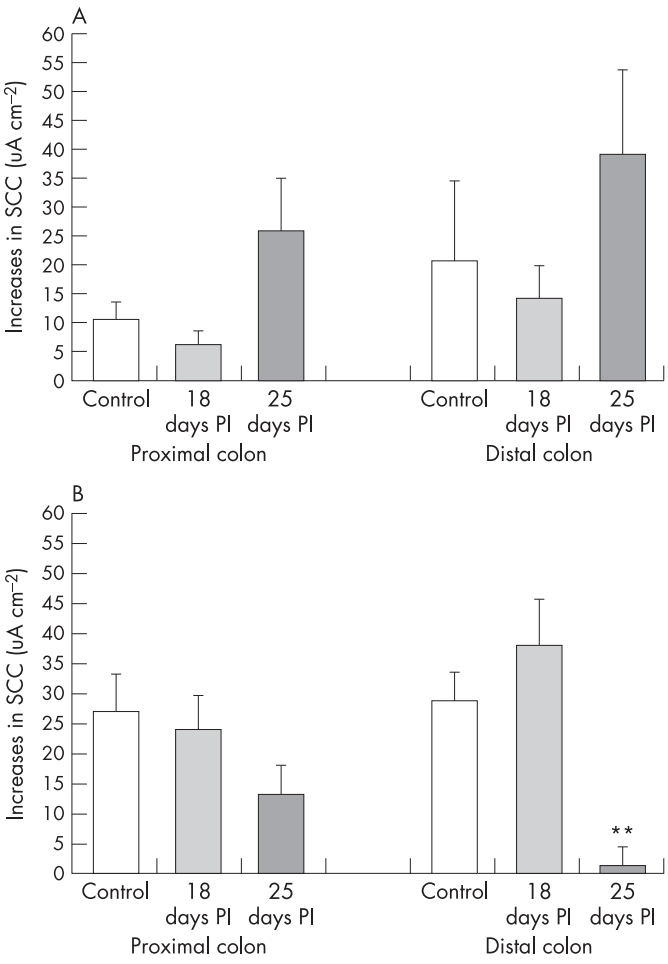

Figure 3 Effects of (A) cholic acid (1 mmol serosally) and (B) taurodeoxycholic acid (TDCA; 1 mmol serosally) on short circuit current (SCC) of stripped proximal and distal colon of non-infected control and *Trichinella spiralis*-infected animals. Results are expressed as mean values ± SEM. **p<0.01 with n = 6 for each tissue sample. PI, Postinfection.

25 postinfection, which was significant in the case of TDCA (p<0.05) partly because TDCA produced a greater SCC response than cholic acid and therefore a greater potential for reduction. In the ileum, however, a significant increase in SCC was observed at 18 days in response to both cholic acid (p<0.01; fig 2A) and TDCA (p<0.01; fig 2B) when compared with controls. By day 25 postinfection, however, this increase was reversed and SCC was significantly lower at day 25 compared with day 18, although this was not significantly below the control value. In Cl^--free conditions, when all Cl^- in both mucosal and serosal solutions had been replaced with gluconate, there were no increases in SCC after either cholic acid or TDCA administration in control or infected mice (data not presented). This confirms previous findings that bile acid-induced increases in intestinal SCC are predominantly caused by Cl^- secretion in mice.[18 19]

Effects of cholic acid and TDCA on ion transport in the colon

Basal SCC

In neither the proximal nor the distal colon was there any significant effect of infection at days 18 and 25 on basal SCC despite a trend towards a decrease at day 18 and an increase at day 25 (fig 1B).

Response to bile acids

In the proximal colon, no significant changes in SCC in response to cholic acid (fig 3A) or TDCA (fig 3B) were observed. In the distal colon, however, a significant decrease in SCC was observed at day 25 postinfection in response to TDCA (p<0.01; fig 3B). Again, preliminary studies using Cl^--free Krebs determined that the increases in SCC were caused by Cl^- secretion (data not presented).

Effects of *T. spiralis* on small intestinal fluid and TCA uptake

MFT in everted jejunal sacs was not different from controls at either timepoint postinfection. In contrast, MFT in ileal sacs was very markedly decreased at both 18 (p<0.001) and 25 days (p<0.01) postinfection when compared with ileal controls (fig 4A). Similarly, the low levels of 3H-TCA uptake in the jejunum remained unchanged postinfection but were markedly reduced in the ileum at 18 days (p<0.001). There was some recovery at day 25 but uptake remained significantly below control levels at this timepoint (p<0.05; fig 4B). Calculation of the T/M ratio, indicative of active TCA uptake when greater than 1, demonstrated that under control conditions bile acid absorption was active in the ileum, but passive in the jejunum

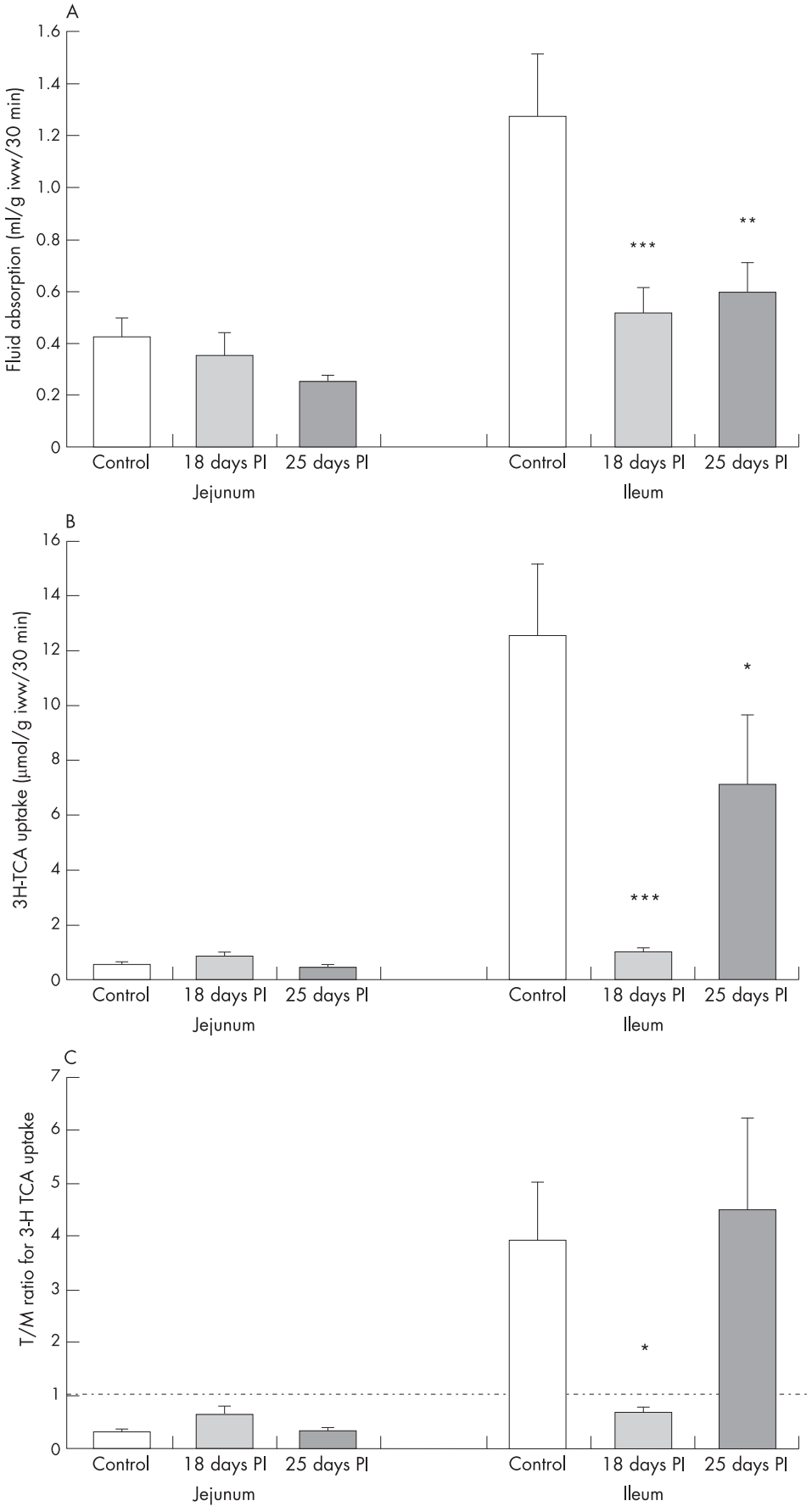

Figure 4 (A) Intestinal fluid absorption (B) tritiated taurocholic acid (3H-TCA) and (C) T/M ratio in jejunal and ileal samples from non-infected control and *Trichinella spiralis*-infected mice. Results are expressed as mean values ± SEM. *p<0.05, **p<0.01, ***p<0.001 with n = 6 for each tissue sample. iww, Initial wet weight.

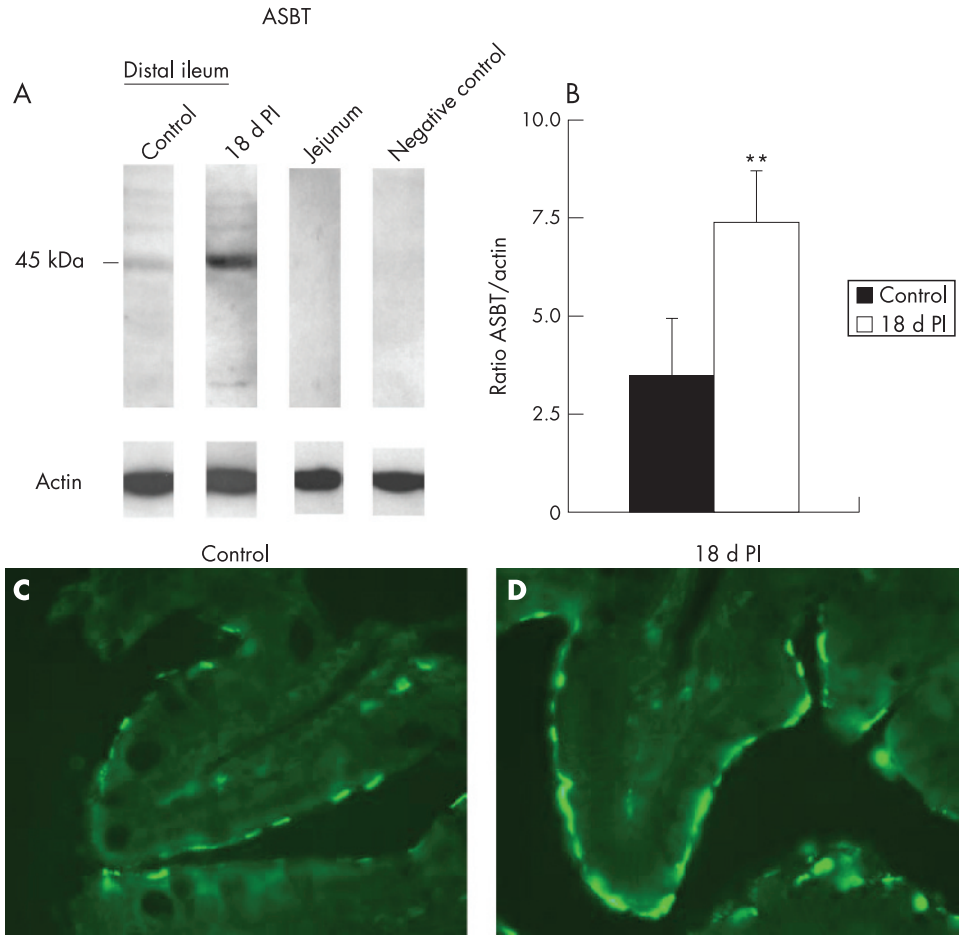

Figure 5 Effects of *Trichinella spiralis* infection on apical sodium-dependent bile acid transporter (ASBT) protein expression. (A) Western blot showing a prominent band of approximately 45 kDa in the distal ileum, which correlates with the monomeric form of the ASBT protein. ASBT expression was not found in the jejunum and in the absence of primary antibody (negative control). (B) Protein expression is increased in the distal ileum of mice 18 days postinfection (PI) with *T. spiralis*. (C, D) ASBT expression at the apical membrane of epithelial cells along the length of the entire villus in the distal ileum. The intensity of staining was greater in the ileum from infected animals (D) compared with uninfected controls (C).

(fig 4C), consistent with the localisation of the ASBT to the terminal ileum.[25] At 18 days postinfection, the ileum exhibited a significantly reduced ($p<0.05$) T/M ratio, which was below unity, suggesting that it had completely lost its ability to transport TCA actively at day 18 postinfection with recovery by day 25.

Effects of *T. spiralis* on the expression of ASBT in the distal ileum

Western blot analysis showed a protein band of approximately 45 kDa for ASBT in the epithelial cells of the distal ileum (fig 5A). ASBT expression in the distal ileum was increased at 18 days postinfection by approximately 113% with respect to control animals (100%; fig 5B). No protein band was found in the jejunum, a negative control.

Immunohistochemistry studies detected ASBT expression at the apical pole of epithelial cells along the length of the entire villus in the distal ileum. ASBT expression in the distal ileum was increased at 18 days postinfection with respect to jejunal control animals (fig 5C, D). No staining was found in the control experiments without the primary antibody.

DISCUSSION

The current study presents novel data demonstrating enhanced ileal ion secretion in response to a primary (cholic acid) and secondary (TDCA) bile acid in a mouse model of postinfectious bowel dysfunction. Furthermore, a striking decrease in predominantly active bile absorption in the ileum was also observed. Bile acids normally undergo enterohepatic circulation by passive and active uptake processes in the jejunum and ileum, respectively. When this circulation is interrupted, however, bile acids enter the colon in increased concentrations where they induce ion secretion resulting in diarrhoea. The current study demonstrated that *T. spiralis* infection also rendered the distal colon insensitive to bile-induced secretion as evidenced by the reduced SCC response to serosally administered TDCA. If similar events also occur in postinfectious patients, then the combined effects of these disturbances may contribute to some of the symptoms observed in PI-IBS patients. These results attain greater significance in the light of emerging evidence that BAM is common in IBS patients.[13] In humans, BAM leads to a compensatory (up to) 15-fold increase in hepatic biosynthesis, thereby allowing increased amounts of bile acids to pass into the colon and induce secretion.[25 26] If a similar increase in bile delivery occurs in these infected mice, the decreased secretion in the distal colon may represent a compensatory mechanism to overcome the elevated level of colonic bile acids after small bowel malabsorption.

The decrease in active bile uptake in a histologically normal (data not presented) ileum is particularly striking and may be of potential clinical significance. A diminished capacity of the ileal mucosa for bile acid uptake plays a significant role in BAM. Ileal absorption of bile acids is mediated primarily by the ASBT.[14 15] Mutations in the *ASBT* gene have been described in Crohn's disease patients. Furthermore, decreased ASBT expression was observed in an experimental model of ileitis.[27–29] We anticipated that the functional change in bile salt uptake would be reflected in downregulation in the expression of ASBT. Both Western blot analysis and immunohistochemistry showed a consistent

increase, however, and not a decrease, in protein in the ileal mucosa of postinfected mice compared with controls. Moreover, the apical distribution of protein revealed immunohistochemically would indicate that the protein is correctly trafficked to the appropriate membrane. ASBT is subject to posttranslational regulation by phosphorylation/dephosphorylation and protein–protein interactions and it is therefore possible that the transport function of the protein has been inhibited after infection.[30] Alternatively, bile salt extrusion from the basolateral membrane could be adversely affected. This would seem unlikely because uptake into the gut tissue itself as well as into the serosal fluid was assessed in the current study and gut tissue content was also significantly reduced in postinfected animals (the data shown graphically are the sum of serosal uptake and gut tissue content). Interestingly, downregulation of the serotonin transporter has been identified in this animal model and so it is possible that transporter function can be affected.[31]

Numerous mechanisms may explain the enhanced basal and bile-stimulated secretory events observed in the current study. Wheatcroft and colleagues[31] recently demonstrated a significant and sustained increase in the number of serotonin/5HT-containing enteroendocrine cells within the gut of *Trichinella*-infected animals. Serotonin is a key mediator of secretion within the gut, and increased postprandial serotonin release has been observed in IBS patients.[32] As mentioned earlier, they also demonstrated a decrease in the serotonin uptake transporter and suggested that more serotonin was therefore available for activity within the mucosa. Mast cell hyperplasia is also a frequent finding after *Trichinella* infection.[31 33] Histamine, released from mast cells in response to bile acids, binds to histamine H_1 receptors on the basolateral membranes of secretory epithelial cells and evokes Cl^- secretion.[16 34] Wheatcroft and colleagues[31] demonstrated a peak increase in mast cell numbers 14 days postinfection, however, and although they did not investigate at timepoints similar to the current study, they did demonstrate that this increase was transient, with normal mast cell numbers observed 56 days postinfection. The contribution of mast cells to the increased secretory events may therefore not be as pivotal as the contribution of enteroendocrine cells. The secretory actions of both cholic acid and TDCA may also be partly mediated via a neural pathway.[21 35] Interactions between the intestinal nervous and immune systems may generate a coordinated response to enteric infection that involves the amplification of intestinal secretion and activation of mast cells and other inflammatory cells, together with an increase in motility.[36]

It is interesting that the initial augmentation of secretion at 18 days postinfection, was reversed by day 25, with bile unable to induce even control levels of secretion. This biphasic influence of the postinfection process on bile-induced secretion in the jejunum and ileum is difficult to explain. It is possible that enterocytes with impaired ASBT function (if any) at day 18 postinfection may have been replaced by the process of continuous epithelial renewal every three to five days with enterocytes with normal ASBT function by day 25 postinfection. It is interesting that despite the restoration of active bile absorption at 25 days (T/M ratio > 1), active absorption still remained significantly less than in control animals. Neuronal remodelling has also been observed in the post-inflamed gut after infection with the nematode *Nippostrongylus brasiliensis*, which may serve to help normalise gut function in the face of disturbed neuro-effector mechanisms.[37]

In addition to enhanced secretion in response to serosally administered bile, the current study demonstrated that the non-stimulated infected ileum was in a hypersecretory state. This is demonstrated by an increased basal SCC in the ileum at 18 days postinfection. It is interesting that despite an increased basal secretion, bile acids can provoke yet further increases in secretion. It is possible that the basal increase is caused by a physiological response of the small intestine to "flush" or expel *T. spiralis* from the digestive tract. The primary habitat of the worm is the jejunum and this segment of the small intestine did not demonstrate any increases in basal SCC. Furthermore, the presence of the worm is usually not demonstrated at the timepoints investigated as it is expelled by day 14 postinfection.[10]

In conclusion, these studies indicate that both bile acid-induced secretion and active bile acid reabsorption are altered in this murine model of postinfective visceral hypersensitivity. Increased secretion can contribute directly to the development of diarrhoea, whereas reduced ileal reabsorption will result in the appearance of bile acids in the colon, leading to a colonic secretory response and hypermotility. These findings are consistent with the observation of BAM in some patients with PI-IBS, and suggest a pathophysiological basis for the diarrhoeal symptoms in this subgroup. Whether the increased secretion is mediated via neural, immune, endocrine or paracrine mediators requires further research, but identifying changes in the expression of ASBT in this model and clinically in PI-IBS patients may prove significant.

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REFERENCES

1. **Spiller RC.** Irritable bowel syndrome. *Br Med Bull* 2005;**72**:15–29.
2. **Thompson WG,** Longstreth GF, Drossman DA, *et al*. Functional bowel disorders and functional abdominal pain. *Gut* 1999;**45**:II43–7.
3. **Crowell MD,** Harris L, Jones MP, *et al*. New insights into the pathophysiology of irritable bowel syndrome: implications for future treatments. *Curr Gastroenterol Rep* 2005;**7**:272–9.
4. **Talley NJ.** Environmental versus genetic risk factors for irritable bowel syndrome: clinical and therapeutic implications. *Rev Gastroenterol Disord* 2005;**5**:82–8.
5. **Gershon MD.** Nerves, reflexes, and the enteric nervous system: pathogenesis of the irritable bowel syndrome. *J Clin Gastroenterol* 2005;**39**:S184–93.
6. **Barbara G,** De Giorgio R, Stanghellini V, *et al*. New pathophysiological mechanisms in irritable bowel syndrome. *Aliment Pharmacol Ther* 2004;**20**:1–9.
7. **Parry S,** Forgacs I. Intestinal infection and irritable bowel syndrome. *Eur J Gastroenterol Hepatol* 2005;**17**:5–9.
8. **Spiller RC.** Postinfectious irritable bowel syndrome. *Gastroenterology* 2003;**124**:1662–71.
9. **Akiho H,** Deng Y, Blennerhassett P, *et al*. Mechanisms underlying the maintenance of muscle hypercontractility in a model of post-infective gut dysfunction. *Gastroenterology* 2005;**129**:131–41.
10. **Barbara G,** Vallance BA, Collins SM. Persistent intestinal neuromuscular dysfunction after acute nematode infection in mice. *Gastroenterology* 1997;**113**:1224–32.
11. **Dunlop SP,** Jenkins D, Spiller RC. Distinctive clinical, psychological, and histological features of postinfective irritable bowel syndrome. *Am J Gastroenterol* 2003;**98**:1578–83.
12. **Wildt S,** Norby Rasmussen S, Lysgard Madsen J, *et al*. Bile acid malabsorption in patients with chronic diarrhoea: clinical value of SeHCAT test. *Scand J Gastroenterol* 2003;**38**:826–30.
13. **Smith MJ,** Cherian P, Raju GS, *et al*. Bile acid malabsorption in persistent diarrhoea. *J R Coll Physicians Lond* 2000;**34**:448–51.
14. **Hofmann AF.** Bile secretion and the enterohepatic circulation of bile acids. In: Feldman M, Scharschmidt BF, Sleisenger MH, eds. Sleisenger & Fordtran's gastrointestinal and liver disease, 6th edn. Philadelphia: W B Saunders Company, 1998;937–948.

15. **Dawson PA,** Haywood J, Craddock AL, *et al*. Targeted deletion of the ileal bile acid transporter eliminates enterohepatic cycling of bile acids in mice. *J Biol Chem* 2003;**278**:33920–7.
16. **Barrett KE,** Keely SJ. Chloride secretion by the intestinal epithelium:molecular basis and regulatory aspects. *Ann Rev Physiol* 2000;**62**:535–72.
17. **Oddsson E,** Rask-Madsen J, Krag E. A secretory epithelium of the small intestine with increased sensitivity to bile acids in irritable bowel syndrome associated with diarrhoea. *Scand J Gastroenterol* 1978;**13**:408–16.
18. **Hardcastle J,** Hardcastle PT, Chapman J, *et al*. Taurocholic acid-induced secretion in normal and cystic fibrosis mouse ileum. *J Pharm Pharmacol* 2001;**53**:711–19.
19. **Hardcastle J,** Hardcastle PT, Chapman J, *et al*. Ursodeoxycholic acid action on the transport function of the small intestine in normal and cystic fibrosis mice. *J Pharm Pharmacol* 2001;**53**:1457–67.
20. **Hardcastle J,** Harwood MD, Taylor CJ. Absorption of taurocholic acid by the ileum of normal and transgenic ΔF508 cystic fibrosis mice. *J Pharm Pharmacol* 2004;**56**:445–52.
21. **Gelbmann CM,** Schteingart CD, Thompson SM, *et al*. Mast cells and histamine contribute to bile acid-stimulated secretion in the mouse colon. *J Clin Invest* 1995;**95**:2831–9.
22. **Bercik P,** Wang L, Verdu EF, *et al*. Visceral hyperalgesia and intestinal dysmotility in a mouse model of postinfective gut dysfunction. *Gastroenterol* 2004;**127**:179–87.
23. **Castro GA,** Fairburn D. Carbohydrates and lipids in *Trichinella spiralis* larvae and their utilisation in vitro. *J Parasitol* 1969;**55**:51–8.
24. **Andres H,** Rock R, Bridges RJ, *et al*. Submucosal plexus and electrolyte transport across rat colonic mucosa. *J Physiol* 1985;**364**:301–12.
25. **Cachefo A,** Boucher P, Dusserre E, *et al*. Stimulation of cholesterol synthesis and hepatic lipogenesis in patients with severe malabsorption. *J Lipid Res* 2003;**44**:1349–54.
26. **Hofmann AF.** Bile acids – the good, the bad and the ugly. *News in Physiol Scie* 1999;**14**:24–9.
27. **Stelzner M,** Hoagland V, Somasundaram S. Distribution of bile acid absorption and bile acid transporter gene message in the hamster ileum. *Eur J Physiol* 2000;**440**:157–62.
28. **Jund D,** Fantin AC, Scheurer U, *et al*. Human ileal bile acid transporter gene ASBT (SLC10A2) is transactivated by the glucocorticoid receptor. *Gut* 2004;**53**:78–84.
29. **Chen F,** Ma L, Sartor RB, *et al*. Inflammatory-mediated repression of the rat ileal sodium-dependent bile acid transporter by c-fos nuclear translocation. *Gastroenterol* 2002;**123**:2005–16.
30. **Trauner M,** Boyer JL. Bile salt transporters: molecular characterisation, function and regulation. *Physiol Rev* 2003;**83**:633–71.
31. **Wheatcroft J,** Wakelin D, Smith A, *et al*. Enterochromaffin cell hyperplasia and decreased serotonin transporter in a mouse model of post-infectious bowel dysfunction. *Neurogastroenterol Motil* 2005;**17**:863–70.
32. **Dunlop SP,** Jenkins D, Neal KR, *et al*. Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. *Gastroenterology* 2003;**125**:1651–9.
33. **Vallance BA,** Blennerhassett PA, Huizinga JD, *et al*. Mast cell-independent impairment of host defense and muscle contraction in *T. spiralis*-infected W/W^{v} mice. *Am J Physiol Gastrointest Liver Physiol* 2001;**280**:G640–8.
34. **Quist RG,** Ton-Nu H-T, Lillienau J, *et al*. Activation of mast cells by bile acids. *Gastroenterology* 1991;**101**:446–56.
35. **Karlstrom L.** Evidence of involvement of the enteric nervous system in the effects of sodium deoxycholate on small-intestinal transepithelial fluid transport and motility. *Scand J Gastroenterol* 1986;**21**:321–30.
36. **Perdue MH,** McKay DM. Integrative immunophysiology in the intestinal mucosa. *Am J Physiol* 1994;**267**:G151–65.
37. **Stead RH,** Kosecka-Janiszewska U, Oestreicher AB, *et al*. Remodeling of B-50 (GAP-43) & NSE-immunoreactive mucosal nerves in the intestines of rats infected with *Nippostrongylus brasiliensis*. *J Neurosci* 1991;**11**:3809–21.

Robin Spiller, editor

Massive haematemesis in an African male

CLINICAL PRESENTATION

A 31-year-old man of African origin presented with haematemesis and melena. He previously was healthy, without a history of non-steroidal anti-inflammatory drug (NSAID) use.

On examination, the patient was pale and tachycardic. Laboratory tests showed haemoglobin of 3 g/dl. Upper gastrointestinal endoscopy, performed after blood transfusion, showed a large bleeding ulcer, 2 cm in width with an irregular margin, on the posterior wall of the duodenal bulb. Gastric mucosa was normal, as was the oesophagus. The bleeding seemed to be controlled, after adrenalin injection, but 2 days later the haematemesis recurred.

Laparotomy revealed a perforated ulcer of the duodenal bulb, penetrating the pancreas, and multiple lymph node enlargement around the duodenum and the greater curvature. A gastrojejunostomy was performed. There were no ascites or peritoneal nodules.

Figure 1 shows an H&E stain of the microscopic section of the duodenal ulcer, and figs 2 and 3 show a microscopic section of a lymph node.

QUESTION

What is the diagnosis?

See page 58 for answer

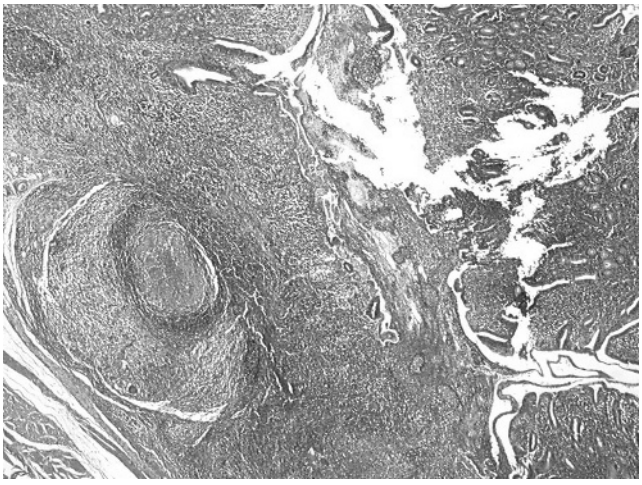

Figure 1 Ulcerated mucosa, with epithelioid granuloma, and central necrosis in the submucosa. H&E, original magnification ×50.

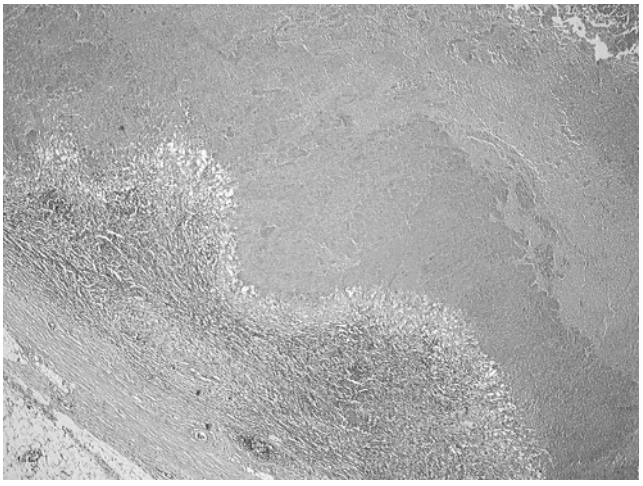

Figure 2 Large area of caseous necrosis in the perigastric lymph node, surrounded by an epithelioid reaction. H&E, original magnification ×50.

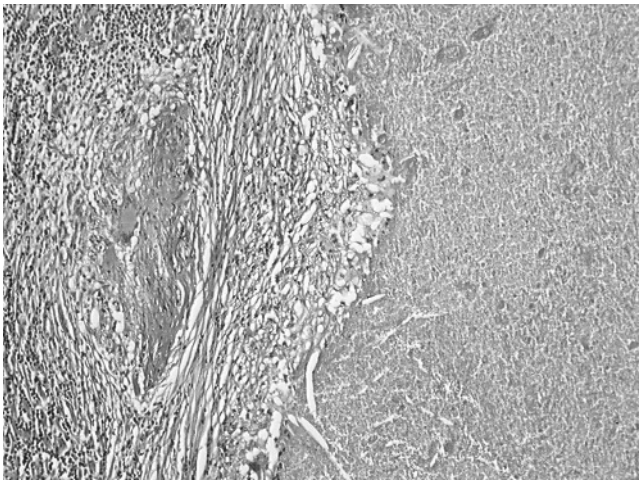

Figure 3 High power view of the epithelioid granuloma in the perigastric lymph node, with Langerhan's giant cell (arrow). H&E, original magnification ×200.

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Intestinal secretory and absorptive function in *Trichinella spiralis* mouse model of postinfective gut dysfunction: role of bile acids

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Competing interests: None.

REFERENCES

1. **Walker-Smith JA,** Guandalini S, Schmitz J, *et al*. Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition. *Arch Dis Childhood* 1990;**65**:909–11.
2. **Hill ID,** Dirks MH, Liptak GS, *et al*. Guideline for the Diagnosis and Treatment of Celiac Disease in Children: Recommendations of the North American Society for Paediatric Gastroenterology, Hepatology and Nutrition. *J Paediat Gastroenterol Nutr* 2005;**40**:1–19.
3. **Kaukinen K,** Collin P, Maki M. Latent celiac disease or celiac disease beyond villous atrophy? *Gut* 2007;**56**:1339–40.

CORRECTIONS

doi:10.1136/gut.2007.135897corr1

Y A Abed, W Hameed, J Roy, *et al*. Appendicitis in an adult patient with cystic fibrosis: a diagnostic challenge (*Gut* 2007;**56**:1799–1800). The first author's name was published incorrectly and should be Y Al-Abed.

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N Kalia, J Hardcastle, C Keating, *et al*. Intestinal secretory and absorptive function in *Trichinella spiralis* mouse model of postinfective gut dysfunction: role of bile acids (*Gut* 2008;**57**:41–9). The list of authors was published in the wrong order: the correct order is N Kalia, J Hardcastle, L Grasa, C Keating, P Pelegrin, KD Bardhan, D Grundy.

Editor's quiz : GI snapshot

ANSWER

From the question on page 604

An excision biopsy of the neck lesion showed fragmented elastic fibres in the middle and deep dermis (fig 1), consistent with pseudoxanthoma elasticum (PXE). Ocular fundus photography demonstrated retinal angioid streaks. Thus, a diagnosis of PXE with colonic involvement was made. PXE primarily affects the elastic fibres, which is characterised by cutaneous and ocular lesions and widespread vascular abnormalities in the various organs. Its most common gastrointestinal presentation is gastric bleeding. PXE is, however, rarely associated with gastric and colorectal cobblestone appearance similar to diffuse xanthomas. There was a suggestive report on deterioration of PXE in a patient with Crohn's disease after steroid therapy.[1] The timed-release form of mesalamine used here (Pentasa) allows for maximal drug delivery in the colon, where it could exert anti-inflammatory effects possibly dependent on peroxisome proliferator-activated receptor-γ against his colonic lesions.

Gut 2008;**57**:716. doi:10.1136/gut.2007.120345a

REFERENCE

1. **Jones AR,** Florin TH. Deterioration of pseudoxanthoma elastica in a patient with active Crohn's disease. *Aust NZ J Med* 1995;**25**:739.

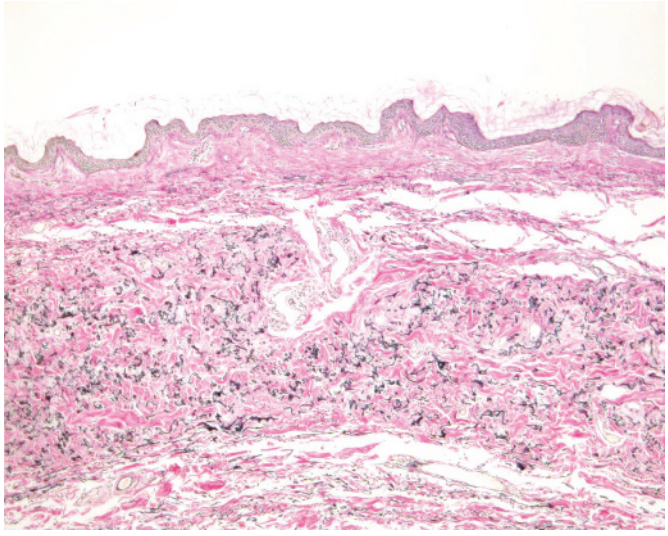

Figure 1 An excisional biopsy specimen of the affected neck skin. Staining of elastic tissue shows fragmented elastic fibres in the middle and deep dermis.