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Macroscopic, microscopic and biochemical characterisation of spontaneous colitis in a transgenic mouse, deficient in the multiple drug resistance 1a gene

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1 A novel animal model of spontaneous colonic inflammation, the multiple drug-resistant (mdr1) $a^{-/-}$ mouse, was identified by Panwala and colleagues in 1998. The aim of our study was to further characterise this model, specifically by measuring cytokines that have been implicated in inflammatory bowel disease (IBD) (IL-8 and IFN-γ) in the colon/rectum of mdr1$a^{-/-}$ mice, and by determining the sensitivity of these, together with the macroscopic, microscopic and disease signs of colitis, to dexamethasone (0.05, 0.3 and 2 mg kg^{-1} subcutaneously (s.c.) daily for 7 days).

2 All mdr1$a^{-/-}$ mice had microscopic evidence of inflammation in the caecum and colon/rectum, while control mice with the same genetic background did not. Significant increases in colon/rectum and caecal weights and also in cytokine levels (both IFN-γ and IL-8) in homogenised colon/rectum were observed in mdr1$a^{-/-}$ mice compared to mdr1$a^{+/+}$ mice.

3 Dexamethasone reduced the increases in tissue weights and also microscopic grading of colitis severity, but had no effect on IFN-γ or IL-8.

4 This study supports the similarity of the gastrointestinal inflammation present in mdr1$a^{-/-}$ mice to that of human IBD, in particular Crohn's disease. This has been demonstrated at the macroscopic and microscopic levels, and was supported further by elevations in colonic levels of IFN-γ and IL-8 and the disease signs observed. The incidence of colitis was much higher than previously reported, with all mice having microscopic evidence of colitis. The limited variance between animals in the parameters measured suggests that this model is reproducible.

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Abbreviations: ANOVA, analysis of variance; CD, Crohn's disease; DSS, dextran sodium sulphate; GI, gastrointestinal; H&E, haematoxylin and eosin; IBD, inflammatory bowel disease; mdr1$a^{-/-}$, multiple drug-resistant 1a deficient; NOD2, nucleotide-binding oligomerisation domain 2; PBMCs, peripheral blood mononuclear cells; s.c., subcutaneously; TNBS, tri-nitrobenzenesulphonic acid; UC, ulcerative colitis

Introduction

Despite significant investigation, the aetiology of IBD is unclear; however some insight into potential disease mechanisms has been obtained from animal models. For example, rodents deficient in genes encoding IL-2 (Sadlack *et al*., 1993), IL-10 (Kuhn *et al*., 1993) and TGF-β (Shull *et al*., 1992) develop intestinal inflammation. Furthermore, selective gene deletion of proteins involved in T-cell function, such as Gαi2 (Rudolph *et al*., 1995) or TCRα (Mombaerts *et al*., 1993), result in spontaneous intestinal inflammation, although in some cases this is linked to *Helicobacter* infection (Berg *et al*., 1998; Kulberg *et al*., 1998; 2001; 2002; Fox *et al*., 1999; Burich *et al*., 2001; Engle *et al*., 2002). These studies have contributed to the hypothesis that the persistent inflammation seen in IBD may be the result of either enhanced or aberrant immunological responsiveness to normal constituents of the gut lumen, or an overall autoimmune dysregulation and imbalance.

Intestinal flora may also be an important co-factor in the pathogenesis of intestinal inflammation. In several rodent models, spontaneous colitis can be prevented if animals are maintained in a germ-free environment (Taurog *et al*., 1994; Dianda *et al*., 1997) and studies have shown that intestinal inflammation can be treated with antibiotics (Panwala *et al*., 1998). In addition, patients with IBD have adverse and enhanced reactivity to their autologous resident flora (MacDonald, 1995). Support for this hypothesis comes from studies using mice with defects in epithelial barrier cell function (Hermiston & Gordon, 1995; Mashimo *et al*., 1996), which have evidence of inflammation in the gastrointestinal (GI) tract. There is evidence to suggest that nucleotide-binding oligomerisation domain 2 (NOD2), which serves as an intracellular receptor for bacterial lipopolysaccharide and is linked to the activation of the transcription factor, NF-κB, is linked to IBD pathogenesis and may play a role in activation

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of inflammatory pathways induced by micro-organisms (Hugot, 2003). Mutations in NOD2 may also play a role in the development of disease in a significant number of CD patients (Amre, 2003). It can be hypothesised that food constituents may contribute to the inflammatory response observed in rodents with defective epithelial cell barriers, since these materials would potentially have access to areas of the gut mucosa, from which they would normally be excluded.

Intestinal epithelial cells (Croop *et al*., 1989; Gottessman & Pastan, 1993) and some subsets of lymphocytes (Bommhardt *et al*., 1994) express mdr genes that encode P-glycoproteins, which serve as drug efflux pumps. These pumps are localised in the plasma membranes of many tissues and appear to play a natural role in protecting the host from potentially harmful compounds. Three mdr1 genes have been identified in rodents (mdr1a, mdr1b and mdr2) and two in humans (MDR1 and MDR2). In mice, mdr1a P-glycoprotein is also expressed in the blood–brain and blood–testis barrier, and mdr1b P-glycoprotein is expressed in adrenal glands, pregnant uterus and ovaries (Croop *et al*., 1989). Both mdr1a and mdr1b P-glycoprotein are expressed in the liver, kidney, spleen and heart (Croop *et al*., 1989).

Mice with a targeted deletion of the mdr1a gene were first generated in 1994. These mice had an increased sensitivity to certain drugs, but did not appear to have any constitutive abnormalities (Schinkel & Smith, 1994). Mdr1a$^{-/-}$ mice are routinely used in studies to evaluate the involvement of P-glycoprotein in the absorption of compounds, brain penetration of drugs and the hepatobiliary excretion of compounds that are potentially substrates for P-glycoprotein. A recent study reported that 25% of mdr1a$^{-/-}$ mice spontaneously developed colonic inflammation that was similar histologically to human IBD (Panwala *et al*., 1998). We have observed similar disease signs and lesions, and preliminary data have been reported (Banner *et al*., 2001). Specifically, disease signs observed included acute body weight loss and soft, 'sticky' faeces, while histopathological analysis revealed a marked, diffuse colitis. The aims of this study were to further characterise the nature of the GI inflammation, determine the similarity of the model to human IBD, as well as its reproducibility and suitability for identifying potential new therapies for IBD. Thus, we monitored disease signs and performed a detailed characterisation of this colitis at the macroscopic and microscopic level, and also investigated the ability of steroid treatment to ameliorate the colitis.

Methods

Animals

A total of 40 male FVB 129P2-Abcb1a^{tm1}N7 (mdr1a$^{-/-}$) mice and six control male mice FVB/NTac (mdr1a$^{+/+}$) from the same genetic background (6–8 weeks old) were obtained from Taconic Farms (U.S.A.). These mice were originally developed by Schinkel & Smith (1994) and were received at Taconic Farms at the 7th backcross (N7) onto the FVB strain. Mice were housed individually in solid bottom cages containing autoclaved Bee Kay bedding (Bantin & Kingman, U.K.) with the addition of Envirodri, a nesting material made from virgin paper, free from deodorising chemicals, to provide enrichment. Mice were fed on RAT & Mouse No. 1 maintenance diet (RMI (E) SQC) (Special Diet services, U.K.). Bottled water and Klortabs (5 ppm) (Arrowmight Biosciences, U.K.) were used to eliminate the possible spread of waterborne bacterial pathogens, and to provide a water supply with constant levels of chlorine (McPerson, 1994). Water was changed twice a week. This study was carried out in accordance with the U.K. legislation and approved by Pfizer's internal ethical review process.

Study design and dosing

Mice (40 mdr1a$^{-/-}$ and 6 mdr1a$^{+/+}$) were acclimatised for 14 days. From a pilot study (Banner *et al*., 2001), growth rate and soft faeces score were found to be predictive of GI inflammation; thus, in the present study, these parameters were monitored. A scoring system was devised with weightings for different disease signs, weighted according to their significance to overall health, and each mouse scored daily. Specifically, soft 'sticky' faeces scored 6, absence of body weight gain scored 1, body weight loss of $\geqslant 0.5\,g\,day^{-1}$ and $\geqslant 20\%$ scored 2 and 6, respectively. The cumulative score was intended to be reflective of colitis severity. Following the acclimatisation period, mdr1a$^{-/-}$ mice with mild and moderate disease scores (mild disease (0–3) was defined by absence of gain or mild body weight loss ($<0.5\,g$) and no other symptoms, whereas moderate disease (4–10) was defined by similar symptoms as a previous group but with soft faeces or greater loss in body weight ($>20\%$)) were randomised into four groups and dosed s.c. with vehicle (water, $n=8$) or dexamethasone ($0.05\,mg\,kg^{-1}$, $n=8$; $0.3\,mg\,kg^{-1}$, $n=9$ or $2\,mg\,kg^{-1}$, $n=8$) daily for 7 days. The 6 mdr1a$^{+/+}$ mice received vehicle (water). During the study period, one mouse from the group treated with $0.3\,mg\,kg^{-1}$ dexamethasone was found dead and three were euthanised (one from the vehicle-treated group, one from the group treated with $0.05\,mg\,kg^{-1}$ dexamethasone and one from the $0.3\,mg\,kg^{-1}$ dexamethasone group). Gross examination was performed, but organs were not processed because of logistic constraints (-related to timings) and/or advanced autolysis. At the end of the study, all mice were euthanised and necropsied. The colon/rectum and caecum from each mouse were weighed and wet weights recorded. Each colon/rectum was divided longitudinally: one specimen was fixed in 10% buffered formaldehyde solution and processed for histological analysis, the other sample was dedicated to cytokine measurement.

Cytokine and protein measurement

Individual colon/rectum samples were homogenised three times for 30 s at 4°C in 3 ml of ice-cold Krebs' solution (Sigma Chemical Co., U.K.). Samples were centrifuged for 15 min at $35{,}000 \times g$ (4°C) and supernatants removed. IL-8 and IFN-γ levels were then measured by ELISA kits (R&D systems, U.K.). The minimum and maximum detection limits of these kits were 31.2 and $2000\,pg\,ml^{-1}$, respectively. Protein levels were assayed in supernatants using the method described by Bradford (1976), utilising Bradford Reagent (Sigma Chemical Co., U.K.) and bovine serum albumin as a standard (Sigma Chemical Co., U.K.).

Histological and morphometric analysis

The colon/rectum was prepared using a 'Swiss roll' technique (Moolenbeek & Ruitenberg, 1981) to evaluate the entire longitudinal section on one slide. Tissues were processed in an automatic tissue processor, embedded in paraffin, sectioned at 4–6 μm, stained with haematoxylin and eosin (H&E), and examined histologically. The colitis was graded by a blinded observer according to the extent and severity of inflammatory changes in mucosal architecture and morphometry. A score of '0' indicated no significant findings, '1' described minimal multi-focal changes involving the apex of the mucosal folds of the proximal colon, '2' indicated mild multi-focal changes localised mainly within the mucosal folds of the proximal colon with minimal involvement of the remaining areas, '3' described marked changes involving diffusely the whole middle and distal colon and rectum with mild multi-focal changes in the proximal colon and '4' described severe changes involving the entire middle and distal colon and rectum, with mild-to-moderate diffuse changes in the proximal colon. The height of the colon/rectum mucosa was assessed with an Aristoplan (Leitz) microscope coupled with an interactive microcomputer-assisted image analysis system (Leica Qwin). Approximately every 100 μm, from the ano-rectal junction to the proximal colon, the operator determined two points (surface and base of mucosal crypts) and the distance between these two points was measured automatically by the calibrated system.

Search for pathogens

A number of pathogens such as *H. hepaticus*, *H. bilis* and *H. rodentium* have been associated with the development of GI inflammation in rodents (Ward *et al*., 1996; Shomer *et al*., 1998; Chin *et al*., 2000; Maggio-Price *et al*., 2002). Three separate techniques were used to confirm the presence or absence of all known murine pathogens in faecal and colon/rectum samples. Faecal samples were analysed using PCR, by two independent collaborators: Charles Clifford, Charles River, MA, U.S.A. and Jeff Needham, The Microbiology Laboratories, Middlesex, England. Colon/rectum samples were analysed histologically using Warthin Starry and Fite Faraco stains and by electron microscopy. Serological and bacteriological studies for other known pathogens were also carried out according to FELASA recommendations (FELASA recommendations, 1994; 1996).

Statistical analysis

Data were analysed for the 29 mdr1a$^{-/-}$ and 6 mdr1a$^{+/+}$ mice that completed the study. Differences between vehicle-treated mdr1a$^{-/-}$ and mdr1a$^{+/+}$ mice were analysed using a two sample *t*-test (assuming equal variance for IFN-γ, growth rate in the acclimatisation period and final body weight, and unequal variance for IL-8, colon/rectum weight, caecal weight, colon/rectum-to-body weight ratio, caecum-to-body weight ratio and growth rate during the study period). The effects of dexamethasone treatment were analysed using analysis of variance (ANOVA). Where treatment effects were found to be significant, 95% confidence intervals were calculated. Histological colitis severity score is an ordered categorical measure, graded on a scale of 0–4. These type of data are generally analysed using a proportional odds model; however, due to the small sample size this was not appropriate, and ANOVA was used. This assumes normally distributed data, which, although we had categorical data, seemed to hold approximately true. All analysis was carried out using Genstat 5 Release 4.2 for Windows.

Results

Symptomatic grading of colitis severity

On arrival, the mean body weight of mdr1a$^{-/-}$ mice was 22.50 ± 0.349 and $22.50 \pm 0.74\,g$ for the mdr1a$^{+/+}$ mice. There was no statistically significant difference in growth rate between mdr1a$^{-/-}$ and mdr1a$^{+/+}$ mice (0.27 vs 0.29 g day^{-1}) during the acclimatisation period; however, during the study period, there was a statistically significant difference between vehicle-treated mdr1a$^{-/-}$ and mdr1a$^{+/+}$ mice. Mdr1a$^{+/+}$ mice continued to gain weight, whereas mdr1a$^{-/-}$ mice lost weight, and at the end of the study (when mice were 9–11 weeks old), mdr1a$^{-/-}$ mice were significantly lighter than mdr1a$^{+/+}$ mice (Table 1). During the study, mean soft faeces scores and standard error of means (s.e.m.) were as follows: 45 ± 8.3 (0 mg kg^{-1}), 48 ± 8.3 (0.05 mg kg^{-1}), 33 ± 8.3 (0.3 mg kg^{-1}) and 27 ± 7.8 (2 mg kg^{-1}). Disease signs were not observed in control mdr1a$^{+/+}$ mice. The cumulative scores and s.e.m. were as follows: 50 ± 8.9 (0 mg kg^{-1}), 56 ± 8.9 (0.05 mg kg^{-1}), 37 ± 8.9 (0.3 mg kg^{-1}) and 32 ± 8.3 (2 mg kg^{-1}). Dexamethasone had no significant effect on growth rate, final body weight, soft faeces score or cumulative scores.

Colon/rectum and caecal weights

A significant increase in colon/rectum (691 ± 69 vs 325 ± 26 mg) and caecal (211 ± 12 vs 165 ± 6 mg) weight was observed in the vehicle-treated mdr1a$^{-/-}$ mice vs mdr1a$^{+/+}$ mice ($P<0.05$). Ratios of colon/rectum- and caecum-to-body weight were also significantly higher in vehicle-treated mdr1a$^{-/-}$ mice (Figure 1). Dexamethasone (2 mg kg^{-1}) reduced absolute colon/rectum weight (457 ± 44 vs 691 ± 69 mg; $P<0.01$) and colon/rectum-to-body weight ratio vs vehicle-treated mdr1a$^{-/-}$ mice, while lower doses of dexamethasone had no significant effect. All doses of dexamethasone reduced absolute caecum weights ($P<0.01$) as well as caecal-to-body weight ratios ($P<0.015$) compared to vehicle-treated mdr1a$^{-/-}$

Table 1 Growth rate over the study period and body weight at the end of the study

	Treatment (dosed s.c.)	*Growth rate (g day^{-1}) (mean±s.e.m.)*	*Body weight at end of study (g) (mean±s.e.m.)*
mdr1a$^{+/+}$	Vehicle	0.15 ± 0.039	27.1 ± 0.55
mdr1a$^{-/-}$	Vehicle	-0.44 ± 0.099*	22.8 ± 0.77*
mdr1a$^{-/-}$	0.05 mg kg^{-1} dexamethasone	-0.43 ± 0.099*	22.2 ± 0.77*
mdr1a$^{-/-}$	0.3 mg kg^{-1} dexamethasone	-0.26 ± 0.099*	22.6 ± 0.77*
mdr1a$^{-/-}$	2 mg kg^{-1} dexamethasone	-0.27 ± 0.093	22.7 ± 0.72*

Two-sample *t*-test: *$P<0.05$ vs mdr1a$^{+/+}$.

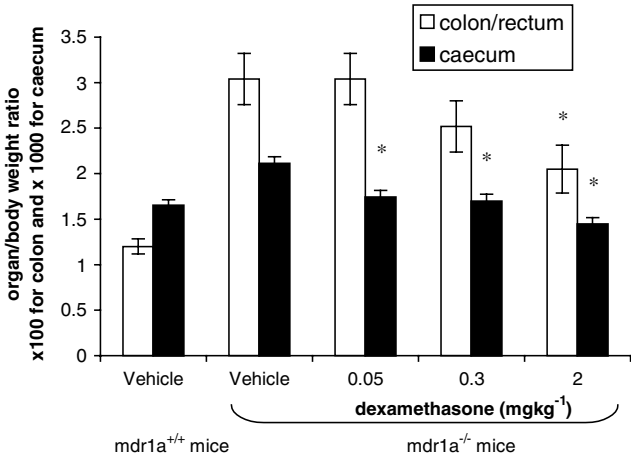

Figure 1 Effect of dexamethasone on colon/rectum- and caecum-to-body weight ratio in mdr1a$^{-/-}$ mice. Data are shown as mean$\pm$s.e.m. *Statistically significant difference compared to the vehicle-treated mdr1a$^{-/-}$ group .Two-sample *t*-test ($P<0.05$).

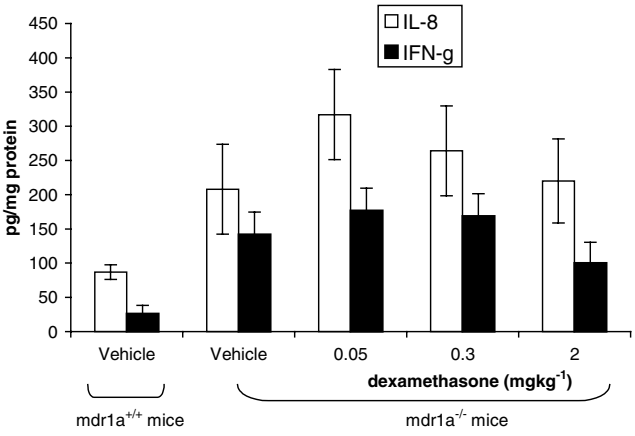

Figure 2 Effect of dexamethasone on IL-8 and IFN-γ in homogenised colon/rectum samples from mdr1a$^{-/-}$ mice. Data are shown as mean$\pm$s.e.m.

mice. The colon/rectum was thickened and oedematous in most mice recorded with grade 3 and 4 inflammation.

Cytokine levels

IL-8 ($P<0.05$) and IFN-γ ($P<0.01$) levels were significantly higher in colon/rectum samples from vehicle-treated mdr1a$^{-/-}$ mice vs mdr1a$^{+/+}$ mice (Figure 2). Dexamethasone had no effect on levels of either cytokine.

Histology

Colon/rectums from mdr1a$^{+/+}$ mice appeared histologically normal (Figure 3a and c). In contrast, colon/rectums from mdr1a$^{-/-}$ mice were characterised by a variable (between different sites in a single mouse using the Swiss Roll technique) degree of infiltration of inflammatory cells within the lamina propria, mainly macrophages, lymphocytes and polymorphonuclear neutrophils, and by mucosal thickening (Figure 3b and d). Increase in crypt lengths, increased

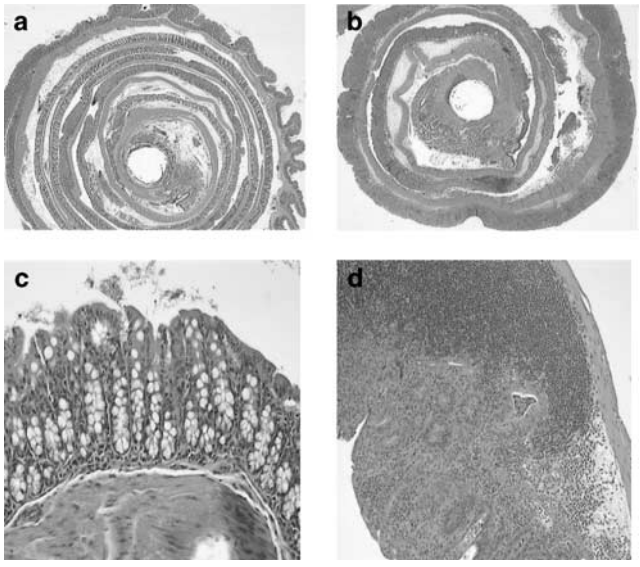

Figure 3 HE-stained sections of mouse colon/rectum from mdr1a$^{+/+}$ (a, c) and mdr1a$^{-/-}$ (b, d) mice at $\times 20$ (a, b) and $\times 200$ (c, d) magnification. Sections taken from an mdr1a$^{+/+}$mouse have a normal appearance, with an abundance of well-differentiated goblet cells (a, c). In contrast, the section from mdr1a$^{-/-}$ mouse shows marked mucosal thickening with goblet cell depletion and a marked proliferative colitis (b, d).

basophilia and number of mitoses of lining enterocytes, goblet cell loss and interstitial oedema were observed. While few erosions were seen in mild colitis (score 2), obliteration of normal architecture, ulceration that varied from superficial to transmural, and crypt abscesses were seen in scoring groups 3 and 4. Colitis appeared to begin in the proximal region of the colon, as this was the main region affected in scoring groups 1 and 2. In severe colitis (scoring groups 3 and 4), lesions were more prominent in the middle and distal regions of the colon and in the rectum.

Severity of colitis score tended to decrease with increasing dose of dexamethasone: 3.3 ± 0.49 ($0\,mg\,kg^{-1}$), 3.6 ± 0.49 ($0.05\,mg\,kg^{-1}$), 2.7 ± 0.49 ($0.3\,mg\,kg^{-1}$) and 2.1 ± 0.46 ($2\,mg\,kg^{-1}$). While there was no statistically significant difference between the treatment means ($P>0.10$), the highest dose of dexamethasone ($2\,mg\,kg^{-1}$) showed some evidence of a statistically significant decrease in colitis severity score compared with the vehicle group ($0.05<P<0.1$).

Morphometry

The morphometric assessment of the height of the mucosa was in agreement with the histological evaluation. Compared to Score 0, increases in mucosal height were similar for scoring groups 1 and 2 (minimal to mild, seen mainly in the proximal region of the colon). However, scoring groups 3 and 4 (marked to severe, prominent in the middle and distal regions of the colon and in the rectum) were markedly different from scoring groups 1 and 2 with a marked thickening of the mucosa (Figure 4). Also of note is the decrease in the length of the colon and rectum as the severity of the colitis increases, a common finding in animal and human colitis. We would like to thank Paul-Robert Mompon for morphometric analysis.

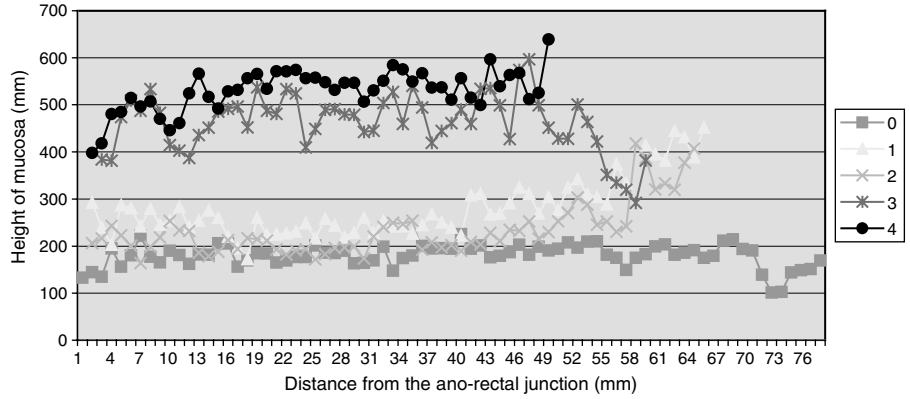

Figure 4 Mucosal height (mm) along the length of the colon/rectum in mdr1a$^{-/-}$ mice. The legend reflects histological grading of colitis from 0–4 (see Methods section for details).

Search for pathogens

The absence of *Helicobacters* (*bilis*, *hepaticus* and *rodentium*) was confirmed by PCR, electron microscopy and histological staining. Absence of other known pathogens including *Citrobacter rodentium* (a bacteria associated with colonic hyperplasia) was confirmed by bacteriological and serological analysis. In summary, mice were free of viruses, bacteria and parasites known to be pathogenic as specified in FELASA recommendations (FELASA recommendations, 1994; 1996).

Conclusion

Whilst building on the observations of the only other report documenting the occurrence of spontaneous gut inflammation in mdr1a$^{-/-}$-deficient mice (Panwala *et al*., 1998), clear differences exist between our study and the earlier one. The differences can be considered in terms of (i) what we have measured (e.g. colonic cytokines), (ii) the technique we used to assess the histology (Swiss Roll technique that enables assessment of the entire longitudinal section of the colon), (iii) nature of treatment examined (steroid), and (iv) our findings, including the extent and pattern of disease.

One difference in our findings is that, in our study, the incidence of colitis was much higher, and its latency much shorter than previously reported. All mice showed microscopic evidence of colitis within 3 weeks of arrival in our facilities (6–11 weeks old), compared to only 25% by 1 year of age in the original study (Panwala *et al*., 1998). The reason for this is unclear and we are not aware of any other reports of colitis spontaneously developing in these mice. In a follow-up study, the same group observed that, if they infected mdr1a$^{-/-}$ mice with *H. bilis,* it accelerated the development of IBD (Maggio-Price *et al*., 2002). A number of pathogens such as *H. hepaticus* (Ward *et al*., 1996; Kulberg *et al*., 1998; 2001; 2002; Chin *et al*., 2000), *H. bilis* (Shomer *et al*., 1998; Maggio-Price *et al*., 2002) and *H. rodentium* (Shomer *et al*., 1998; Burich *et al*., 2001) have been associated with the development of GI inflammation in rodents. Despite extensive diagnostic analysis, we have failed to demonstrate the presence of any known pathogen, suggesting that this is unlikely to be the cause or an exacerbating factor in the development of the colitis in the present study. This does not rule out the possibility of a pathogen, which has yet to be identified, being responsible for causing the increased incidence of colitis observed. Another possible explanation could be differences in animal husbandry, given that the presence of stressors has been linked with both inhibition of the onset and contrastingly, also, induction of inflammatory disease processes (Harbuz *et al*., 1997).

The human MDR1 gene maps to chromosome 7, which has been identified as one of the susceptibility loci associated with human IBD (Satsangi *et al*., 1996). The relevance of an experimental model based on the deletion of the mdr1a gene to human IBD is strengthened by the observed decreased expression and function of MDR1 in intestinal tissues from patients with ulcerative colitis (UC) (Yacyshyn *et al*., 1999). Susceptibility to IBD is consistent with the hypothesis that a lower expression level of MDR1 offers less protection from the accumulation of food constituents and bacteria in the intestine. Indeed, it is interesting to note that mdr1a$^{-/-}$ mice showed equal induction to tri-nitrobenzenesulphonic acid (TNBS) colitis, while in dextran sodium sulphate (DSS) colitis a more severe disease was observed in mdr1a$^{-/-}$ mice (ten Hove *et al*., 2002). Given that T lymphocytes are thought to mediate TNBS colitis, whereas DSS colitis is thought to be due to a direct cytotoxic effect of DSS on epithelial cells; this suggests that mdr1a has an important role in intestinal epithelial resistance to exogenous injury. Further evidence that mdr1 plays a critical role in the GI tract and in the maintenance of homeostasis is discussed in a recent review by Ho *et al*. (2003).

To date, 28 single-nucleotide polymorphisms have been identified in the MDR1 gene (Sakaeda *et al*., 2002). A polymorphism in exon 26, position 3435 (C3435T), which caused no amino-acid change, affected the expression level of MDR1 protein in the duodenum, and thereby intestinal absorption of digoxin, a substrate for MDR1 (Hoffmeyer *et al*., 2000). Recent studies have demonstrated that there is a higher frequency of the C3435T genotype in patients with UC compared with control subjects (Schwab *et al*., 2003), whereas other studies have been more equivocal on the association (Glas *et al*., 2004) or have suggested that the C3435T MDR1 gene polymorphism is not associated with susceptibility for UC (Croucher *et al*., 2003; Gazouli *et al*., 2004). A separate report has suggested that there was a significant association of Ala893 polymorphism within a subset of CD patients (Brant *et al*., 2003).

Previous studies have implicated a number of environmental or dietary constituents as contributing to or exacerbating intestinal inflammation. For example, in rodent models of

colitis induced by DSS, dietary iron was shown to increase disease activity (Reifen *et al*., 2000; Seril *et al*., 2002). Studies in the cotton-top tamarin model of spontaneous colitis have implicated generic environmental factors, specifically temperature, in the onset of colitis in this species (Stonerook *et al*., 1994). Human IBD is characterised by cramping abdominal pain, diarrhoea (which may be bloody), fever and weight loss (Knigge, 2002). In our study, we used a composite index of disease signs that included elements related to features of human disease, in order to provide a measure of the severity of colitis for each mouse. While we observed soft, sticky faeces and other signs of disease in mdr1a$^{-/-}$ mice, the most consistent sign in our study was a reduction in growth rate.

IBD is comprised mainly of UC and CD. UC affects the rectum and colon with continuous, superficial inflammation restricted to the lamina propria and submucosa. In CD, the inflammation can be transmural, is discontinuous, may involve any part of the GI tract and can be associated with the formulation of granulomas. All mdr1a$^{-/-}$ mice in our study had inflammation present in the colon/rectum and caecum, which could be quantified at the macroscopic (by increases in colon and caecal weight) and histological levels. The transmural nature of the most severe inflammation, together with the presence of crypt abscesses, reveals some similarities with human CD. The 'Swiss Roll technique' utilised in our study has the advantage of enabling an assessment of the whole longitudinal section of the colon, rather than just a small section of tissue. This enabled us to establish that colitis appeared to begin within the proximal region of the colon, as this was the region that was always affected in the animals with mild disease. When more severe inflammation was present, lesions were more prominent in the middle and distal regions of the colon and in the rectum. Also of note was the decrease in the length of the colon/rectum as the severity score of the colitis increases, which is a classical finding of colitis. In addition, we observed inflammation in the small intestine of some of the mdr1a$^{-/-}$ mice in the study (data not shown). Thus, our study suggests that the intestinal inflammation in the mdr1a$^{-/-}$ mouse resembles CD in its distribution pattern, especially given that inflammation has been identified at the ileo–caecal junction of over 40% of Crohn's patients (Farmer *et al*., 1976; Okada *et al*., 1987). Our findings are different from the original report that suggests that the inflammation is similar to UC in terms of its restriction to the colon and its superficial nature. The reasons for these differences are not clear. Another similarity between our observations in the mdr1a$^{-/-}$ mouse and those made in patients with IBD is the incidence of extra-intestinal manifestations of inflammation. In the present study, we observed mild inflammation in both the kidney and the liver of mdr1a$^{-/-}$ mice, together with a more significant level of inflammation in the spleen of most of the mice studied, observations that parallel those made in other models of murine colitis (Banic *et al*., 1993). Interestingly, these are known sites of expression of the mdr1a gene product (Thiebaut *et al*., 1987). In IBD patients, extra-intestinal manifestations of disease are well described and, while inflammation of the joints, mouth and eye are the most prevalent of these, cases of nephritis and primary sclerosing cholangitis have been reported, although these tend to occur more commonly in UC than in CD.

The nature of the inflammatory response in IBD has been the focus of much research and debate. Analysis of the types of immune response ongoing in the inflamed intestine of patients has revealed that in CD there is a predominantly T-helper cell type 1 response, with exaggerated production of cytokines such as IFN-γ and IL-12 (Neurath *et al*., 2002), whereas in UC the lesion seems to resemble an antibody-mediated hypersensitivity reaction (Farmer *et al*., 1976; Okada *et al*., 1987; Montelone & Macdonald, 2000; Agnholt & Kaltoft, 2001; McCormack *et al*., 2001). However, downstream inflammatory events appear much the same in both conditions. For example, in both UC and CD, tissue levels of TNF-α, IL-6 and IL-8 are all elevated (Ishiguro, 1999; Katsuta *et al*., 2000; Agnholt & Kaltoft, 2001; McCormack *et al*., 2001) and correlations between IL-8 mRNA expression and degree of colonic inflammation in UC have also been demonstrated (Katsuta *et al*., 2000). In our study, we measured two cytokines, IFN-γ and IL-8, both of which we found to be elevated in mdr1a$^{-/-}$ mice compared to their controls. This is similar to the report published by Maggio-Price *et al*. (2002), in which a significant increase in mRNA for IFN-γ was observed in mdr1a$^{-/-}$ mice compared to mdr1a$^{+/+}$ mice. The results from our study (and Maggio-Price study), documenting elevations in IFN-γ in mdr1a$^{-/-}$ mice thus provide further support for a similarity of this model to human CD. Another difference between our study and that of Panwala is the fact that we examined the effect of steroid treatment in this model of colitis, whereas Panwala examined the effect of a combination of four different antibiotics (a paradigm that is not commonly employed for the treatment of human disease). For most patients, steroids currently represent the most effective drugs for the management of acute flare-ups in moderate to severe IBD (Robinson, 1998). Many studies have investigated the effect of steroid treatment in animal models of GI inflammation. Some have demonstrated efficacy, while others have suggested that steroid treatment may actually worsen disease progression. For example, in a rabbit model, methylprednisolone treatment reduced inflammatory cell recruitment to the colon, but failed to inhibit ulceration (Anthony *et al*., 1997). In separate studies, dexamethasone administered intrarectally was effective at ameliorating TNBS-induced colitis in rat (Palmen *et al*., 1998) and oral administration of dexamethasone was shown to be effective in a guinea-pig model of GI inflammation induced by carageenan (Friend *et al*., 1991). Other studies, however, have shown that dexamethasone cannot prevent induction of colitis and can actually worsen it (van Meeteren *et al*., 2000). These data are largely in accordance with clinical data, as in many patients steroid treatment results in clinical remission that is not accompanied by endoscopic remission, mucosal healing or a reduction in elevated tissue cytokine levels (Beattie *et al*., 1996; Ishiguro, 1999; McCormack *et al*., 2001). In our study, dexamethasone treatment reversed enlargement of the colon/rectum and caecum significantly, but failed to attenuate the elevated levels of either IL-8 or IFN-γ observed in colon/rectum homogenates from mdr1a$^{-/-}$ mice. A study found that patients with CD had a significant decrease in the corticosensitivity of their leukocytes, dexamethasone being less effective at suppressing LPS-stimulated TNF-α, IL-6 and IL-1β release from whole-blood cell cultures from patients with CD compared to healthy volunteers (Franchimont *et al*., 1999). While the reason for this is unclear, it was suggested that this may be related to a specific genetic/constitutional background and/or that it could be

acquired due to inflammation-related endocrine and/or immune factors. A separate study demonstrated that, while there was a systemic increase in the expression of glucocorticoid receptors on peripheral mononuclear cells from IBD patients, there was a decreased binding affinity of glucocorticoids to these receptors (Schottelius *et al*., 2000), suggesting that this could be a factor responsible for this reduced corticosensitivity. This phenomenon may also have been replicated in our study, although we did not specifically investigate this. It is interesting to note that high mdr1 expression was observed on peripheral blood mononuclear cells (PBMCs) and mucosal cells in IBD patients who failed medical therapy (Farrell *et al*., 2000) and that expression of mdr1 mRNA in PBMCs of UC patients who have experienced glucocorticosteroid therapy was significantly greater than that in PBMCs of UC patients with no history of glucocorticosteroid therapy (Hirano *et al*., 2004).

In summary, this study supports the similarity at the macroscopic and microscopic levels between the spontaneously occurring GI inflammation in the mdr1a$^{-/-}$ mouse with that of human IBD, particularly CD. Limited variance between animals in the parameters measured suggests that this model is reproducible. Prior to evaluation of novel therapeutic agents, it will be key to confirm that disease signs and inflammation are responsive to therapeutic agents that are used to treat human disease.

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