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Intestinal inflammation induces adaptation of P-glycoprotein expression and activity

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Abstract

Recent studies suggest that P-glycoprotein (Pgp) encoded by MDR1 gene, may be an important factor in the pathogenesis of inflammatory bowel disease (IBD). In this study, we investigated intestinal Pgp expression and activity: (1) in IL10 deficient ($IL10^{-/-}$) mice which spontaneously develop intestinal inflammation affecting the small and large intestine and (2) in DSS (dextran sodium sulfate)-induced rat colitis.

In $IL10^{-/-}$ enterocolitis mice, rhodamine 123 efflux was reduced by two to three-fold along the small and large intestine. This decrease was associated with a reduction in membrane's Pgp protein levels. A similar three-fold decrease in Pgps activity and expression was observed in the proximal colon in DSS-induced colitis in rats. However, in the non-inflamed ileum in DSS-induced rat colitis, epithelial cell's Pgp activity and protein levels were unexpectedly increased. This effect was specific to local inflammation since LPS induced systemic inflammation did affect neither the intestinal rho 123 efflux transport nor the abundance of the Pgp protein.

These data demonstrate for the first time, an impaired function of epithelial Pgp in IL10 deficient enterocolitis mice. They also show an increase in Pgps activity in the non-inflamed ileum in the DSS-induced rat colitis, which may represent an adaptive mechanism to compensate the impaired activity of Pgp in the colon.

Keywords: Pgp; Colitis; DSS; $IL10^{-/-}$ mice

1. Introduction

P-glycoprotein (Pgp), which is encoded by human MDR1 gene and murine *mdr*1a and *mdr*1b genes, has been studied primarily with respect to its ability to induce a multidrug resistance phenotype. The term multidrug resistance describes the phenomenon of cross-resistance that develops in tumor cells, to a wide range of cytotoxic agents after exposure to a single cytotoxic agent. Pgp is an ATP-dependant membrane transporter which is expressed, in addition to tumor cells, in normal tissues such as the proximal tubule of the kidney, the biliary hepatocytes, the intestinal epithelial cells and the intraepithelial lymphocytes (IEL) of the intestine. In the intestine, Pgp is present on the apical membrane of mature epithelial cells, where it pumps xenobiotics from the inside of cells back into the intestinal lumen [1]. MDR mRNA levels have been shown to increase longitudinally along the intestine in healthy volunteers, with lowest levels in the stomach and highest levels detected in the colon [2]. This distribution of MDR mRNA is in agreement with data showing that Pgp protein increases from the proximal (duodenum) to the distal (ileum) regions of human small intestine [3]. Moreover, the expression of MDR mRNA along the gastrointestinal tract has been negatively correlated to the plasma concentration of a Pgp substrate [2]. Drug absorption is primarily associated with the small and not the large intestine which leaves the physiological role of Pgp in the colon unclear.

On the other hand, *mdr*1a knockout ($mdr1a^{-/-}$) mice are susceptible to spontaneously develop a severe, intestinal inflammation when maintained under specific pathogen-free animal facility conditions, arguing for a role of Pgp in intestinal inflammation [4]. The development of colitis in

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*mdr*1a$^{-/-}$ mice requires a commensal bacterial flora and can be prevented or even reversed by oral treatment with broad-spectrum antibiotics that eliminate intestinal flora. This suggests that one putative function of intestinal Pgp might be to prevent the entry of bacterial toxins into the gut wall's mucosa.

Inflammatory bowel disease (IBD), Crohn's disease and ulcerative colitis are all syndromes characterized by chronic inflammation of the gastrointestinal tract with an etiology which remains unknown. Several polymorphisms of the MDR gene have been described, among which the genomic variant (C3435T) and the Ala893Ser/Thr have received considerable attention. Indeed, higher frequency of the 3435TT genotype or the Ala893 allele of the MDR gene, which are both associated with lower intestinal Pgp expression, was identified by case-control analysis in patients with ulcerative colitis [5] or in patients with Crohn disease and IBD [6]. Thus, the hypothesis that an impairment of the barrier function in 3435TT and Ala893 allele subjects could render this genotype more susceptible to develop IBD is in good agreement with the mdr1a$^{-/-}$ phenotype.

However, whether Pgps expression and function within the mucosa are altered as a function of the degree of inflammation remained to be elucidated.

In this study, we investigated the expression and the activity of Pgp along the intestine during colonic histopathology induced by dextran sodium sulfate (DSS), in IL10 deficient (IL10$^{-/-}$) mice which spontaneously develop intestinal inflammation affecting the small and large intestine and during systemic inflammation induced by LPS (lipopolysaccharide) in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 200–250 g, IL10 deficient mice (IL10$^{-/-}$) and wild-type littermate controls (C57BL6) (Charles River, L'Arbresle, France) were caged under standard laboratory conditions with tap water and regular chow provided ad libitum, in a 12 h/12 h light/dark cycle, at a temperature of 21–23 °C.

IL10$^{-/-}$ mice were periodically examined until clinical signs of intestinal inflammation (diarrhea, melena) occurred (approximately 3 months after birth) and were then used for experiments. The animals were treated in accordance with the European Committee Standards concerning the care and use of laboratory animals.

2.2. Experimental induction of colitis

Colonic inflammation was induced by treatment with drinking water supplemented with 5% (w/v) DSS (mol wt 40,000, ICN Biomedicals, Aurora, OH) for 7 days followed by 5 days of tap water as described previously [7]. Matched control rodents were given only tap water during this period. Animals were weighed daily and monitored for the appearance of diarrhea and blood in the stools. A disease activity index (DAI) was determined by applying a score system as previously described [8].

Acute systemic inflammation was achieved by intraperitoneal injection of 5 mg/kg of LPS (from *E. coli* serotype 055:B5, Sigma Chemicals, St. Louis, MO, USA). Control rats received saline buffer.

2.3. Histologic scoring of inflammation from DSS rats

Rings of ileum, proximal and distal colon were fixed in 10% formalin, samples were processed by routine techniques before embedding in paraffin for histological analysis. Sections (4 μm) were mounted on glass slides, stained with hematoxylin-eosin (H&E) to reveal structural features and examined by light microscopy. The histological score was assessed in accordance with established criteria modified by Barbier et al. [9].

2.4. Determination of myeloperoxidase (MPO) activity

The activity of intestinal (ileum and distal colon) MPO, an enzyme marker of the PMN primary granules, was assayed to monitor the degree of inflammation using the method of Krawisz et al. with minor modifications [10]. Briefly, intestinal tissue samples (∼50–100 mg) were homogenized on ice by using a polytron (13,500 rpm for 1 min) in a solution of 0.5% hexadecyltrimethylammonium bromide (Sigma, St. Louis, MO) in a 50 mM potassium phosphate buffer (pH 6.0, 1 ml/50 mg tissue). The resulting homogenate was subjected to three rapid freezing (−70 °C) and thawing (immersion in warm water, 37 °C) cycles. The samples were then centrifuged (4000 rpm, 15 min, 4 °C) to remove insoluble material. The MPO-containing supernatant (0.1 ml) was assayed spectrophotometrically after the addition of 2.88 ml of phosphate buffer (50 mM, pH 6.0) containing 0.167 mg/ml *o*-dianisidine hydrochloride (Sigma, St. Louis, MO) and 0.0005% hydrogen peroxide. The kinetic of absorbance changes at 470 nm was measured. One international unit of MPO activity, defined as the quantity of enzyme able to convert 1 μmol of hydrogen peroxide to water in 1 min at room temperature, was expressed per gram of tissue.

2.5. Rhodamine 123 efflux assay using everted sac method

Control and DSS-treated rats were sacrificed and the intestine extending from the pyloric end to the rectum was carefully excised. Duodenal, jejunal, ileal, proximal and distal colon segments were immediately chilled and flushed with KRB buffer (117.6 mM NaCl, 25 mM $NaHCO_3$, 1.2 mM $MgCl_2$, 1.25 mM $CaCl_2$, 11 mM Glu-

cose and 4.7 mM KCl, pH 7.4). Fat and mesenteric attachments were removed and the segments everted. Everted sacs of 5 cm in length were filled with 0.8 ml KRB containing 270 μM rhodamine 123 (Sigma–Aldrich, St. Louis, MO, USA), then incubated in 30 ml KRB buffer gassed by 95% O_2/5% CO_2 at 37 °C for 100 min in a shaker bath. Transport of rhodamine 123 from the serosal to the mucosal side was measured by sampling 500 μl of the outer medium every 20 min up to 100 min.

The effect of verapamil (300 μM) (Sigma–Aldrich, St. Louis, MO), a well known Pgp inhibitor, added to the mucosal and serosal sides was determined on rhodamine efflux from the ileum of control and DSS-treated rats.

Rhodamine 123 efflux was similarly monitored on intestinal segments (duodenum, jejunum, ileum, colon) from IL10 knockout mice and their littermate control.

Fluorescence intensity was monitored with a photon technology instrument spectrofluorometer (Bioteck Kontron), detection was set at 500 and 525 nm for excitation and emission, respectively.

In some experiments, Mannitol ^{14}C (0.2 μCi) (Amersham Biosciences, Buckinghamshire, UK) was inserted in the intestine everted sac to assess paracellular transport. Non-radioactive mannitol (20 μM) was added to the mucosal compartment to maintain osmotic balance. Transport of this marker from the serosal to the mucosal side was measured by collecting 500 μl of the external medium every 20 min up to 100 min, and counting it in a β counter.

2.6. Western blot analysis of membrane Pgp

After laparotomy, the ileum and the proximal colon of control and DSS-treated rats, the ileum and the liver from control and LPS treated rats, as well as all the different intestinal segments of $IL10^{-/-}$ and wild type mice were quickly removed and washed with KRB buffer. Mucosa was scrapped on ice and homogenized using a glass potter in a buffer containing 250 mM sucrose, 50 mM Tris–HCl, pH 7.4, with 1 mM PMSF and 0.1 U/ml aprotinin as a protease inhibitor. The homogenate was centrifuged 10 min at 3000 × *g*, and the supernatant was once again centrifuged for 30 min at 15,000 × *g*. The pellet containing the crude membranes was resuspended in a buffer containing 50 mM Mannitol, 50 mM Tris–HCl, pH 7.4, 1mM PMSF and 0.1 UI/ml aprotinin and stored at −80 °C until use. Protein concentration was quantified using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA).

Twenty micrograms of proteins were solubilized in electrophoresis sample buffer containing β-mercaptoethanol and separated on 7.5% SDS-PAGE gel. Resolved proteins were transferred to nitrocellulose membranes and subjected to immunoblot analysis. The blots were blocked 1 h in TBS buffer containing 0.05% Tween 20 (TTBS) and 10% non-fat dry milk. After washing with TTBS, the blots were incubated overnight at 4 °C with 1:200 dilution of monoclonal antibody C219 (Dako, Glostrup, Denmark) or with 1:5000 dilution of antibody anti-β actin clone AC74 (Sigma–Aldrich, St. Louis, MO). After washing five times for 10 min in TTBS, they were further incubated 1 h at room temperature with an anti-mouse horseradish peroxidase-conjugated antibody diluted 1:10,000 (Amersham, UK). The membranes were washed five times for 10 min in TTBS and then probed using chemiluminescence system (ECL, Amersham, UK). The intensity of the bands was quantified using Scion Image (NIH, Scion Corporation, Bethesda, USA)

2.7. Statistic analysis

Results are expressed as mean ± S.E.M. The statistical significance was determined using a one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison test. Differences were statistically significant with $P < 0.05$.

3. Results

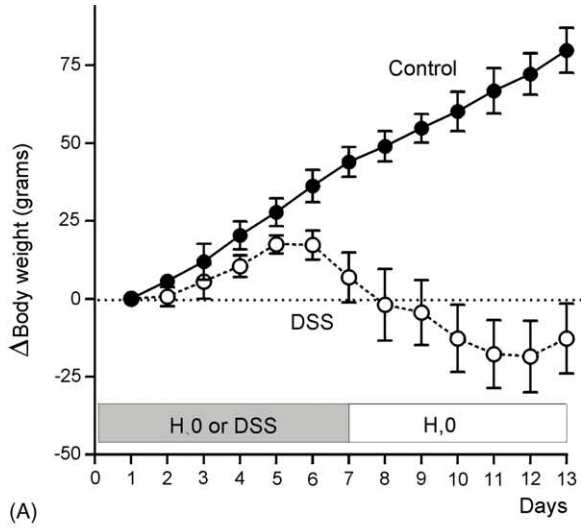
3.1. Induction of colitis in rats

Typically, animals treated with DSS exhibited a progressive decrease in body weight (Fig. 1A) and an increase in disease activity (diarrhea, bloody feces). The colon's length was significantly shortened in DSS-rats ($P < 0.001$ versus control) and tissue-associated MPO activity, an index of neutrophil accumulation, showed a 2.7- and a 1.7-fold increase in distal colon ($P < 0.01$ versus control) and proximal colon, respectively ($P < 0.05$ versus control) (Table 1).

Histological examination revealed a severe disruption of the distal colonic epithelium architecture and a loss of normal crypt architecture in DSS rats. These severe alterations were associated with edema, immune cells infiltrate and ulcerations, sometimes with a complete epithelial denudation (Fig. 1B). The proximal colon of DSS-treated rats was characterized by moderate inflammation with mild edema compared to control (Fig. 1C). Finally, in the ileum of DSS rats, there were no macroscopic or histological signs of inflammation (Fig. 1D) which is in agreement with the unchanged tissue-associated MPO activity (Table 1). Collectively, these data showed that DSS treatment induced colitis with focal inflammation only in the large but not, in the small intestine as it was previously reported [7].

3.2. Intestinal Pgp function in DSS-induced colitis in rats

The functionality of Pgp was evaluated by studying the efflux of rhodamine 123, known as a specific Pgp substrate, using the everted intestine method. The transport



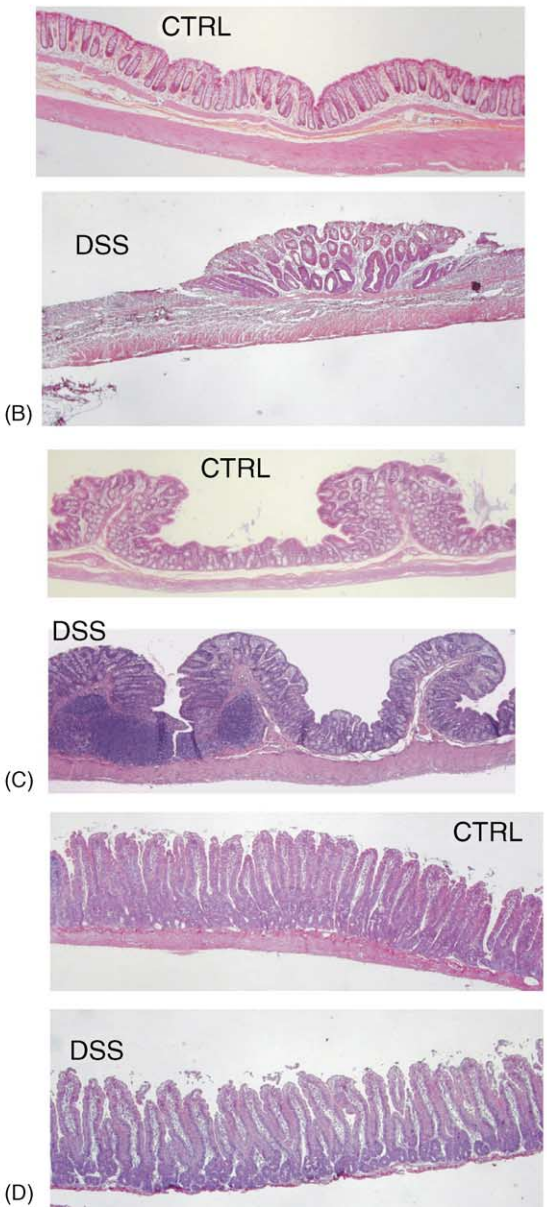


Fig. 1. (A) Change in body weight during DSS treatment. Data are expressed as the mean ± S.E.M. of body weight changes in each animal relative to the weight at the beginning of the experiment in each group. B, C and D Representative photomicrographs of H&E-stained paraffin sections of distal (B), proximal colon (C) and ileum (D) from rats treated with water (CTRL) or with 5% DSS.

Table 1
Changes in different items of the colitis score and in the biological parameters of DSS-treated and control-treated rats

	Control	DSS-treated
Colon length (cm)	21.3 ± 0.3	13.9 ± 0.67***
Clinical scores	0.20 ± 0.01	1.6 ± 0.35***
Histological score	0.75 ± 0.25	15.7 ± 1.9***
MPO activity (UI/g)		
Distal colon	1.7 ± 0.2	4.6 ± 0.5**
Proximal colon	1.8 ± 0.5	3.2 ± 0.8*
Ileum	1.1 ± 0.3	1.0 ± 0.3

The disease activity index and histological score were assessed in accordance with established criteria modified by Barbier et al. [9]. The results are expressed as mean ± SEM of 10 rats in each group.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$ vs. control.

of rhodamine 123 from the serosal to the mucosal side of rat's small and large intestine was investigated between 20 and 100 min, the interval during which rhodamine transport is known to be linear [11].

In control rats, rhodamine efflux increased with a positive scale along the small intestine from the duodenum (0.047 ± 0.015% rho 123 flux/min) to the ileum (0.170 ± 0.021% rho 123 flux/min), and decreased gradually in the large intestine with a minimal secretory flux in the distal part of the colon (0.031 ± 0.005% rho flux/min) (Fig. 2a).

In the ileum of DSS-induced colitis, a significant two-fold increase in rhodamine 123 efflux was observed, whereas in the proximal colon a significant three-fold decrease occurred as compared to control. These modifications in rhodamine efflux were not due to an increase in paracellular permeability since 14C mannitol transport was not modified in the ileum and in the proximal colon of DSS-rats as compared to control (Fig. 2b). In the distal colon, the rhodamine 123 efflux was too low to see any further reduction upon DSS treatment. Moreover, the distal colon of DSS-treated rats exhibited a dramatic increase in mannitol 14C flux (Fig. 2b) arguing for an increased paracellular permeability, as already described [12]. These data, in line with the observed gross alterations of the distal colonic epithelium (Fig. 1), prompted us to exclude the distal colon from further analyzes of Pgp function.

Moreover, the increase of rhodamine efflux in the ileum of DSS-induced rat colitis, was Pgp dependent since it was completely reversed by the addition of verapamil, a well known Pgp inhibitor [11] (Fig. 2c).

The acute systemic inflammation induced by LPS was not associated with any modifications in rhodamine efflux in any of the intestinal segments studied (Fig. 2a). This suggests that the changes in Pgp function in DSS-induced colitis in rats may be specific to focal intestinal inflammation.

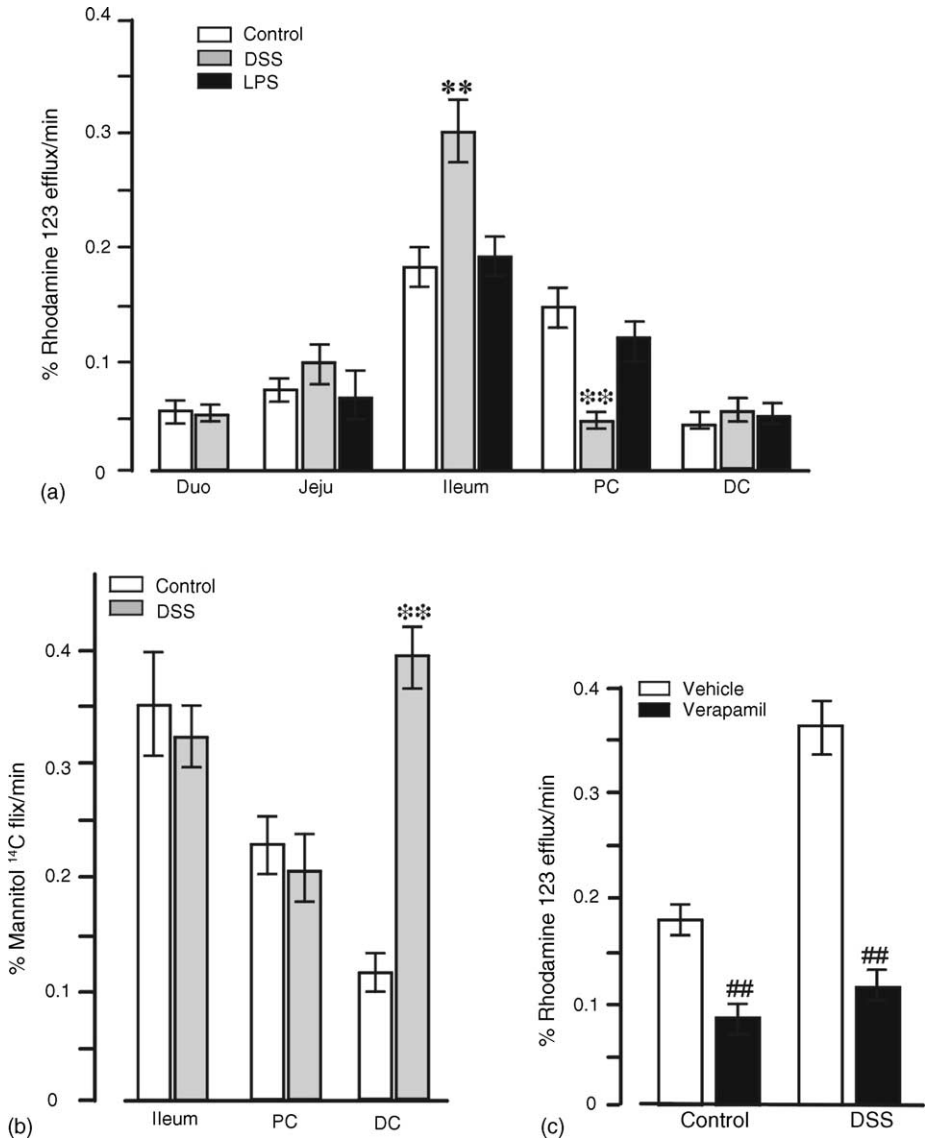


Fig. 2. Intestinal Pgp activity in oral DSS- and systemic LPS-treated rats. (a) Ex vivo transport of rhodamine 123 from serosal to mucosal surfaces across duodenum (Duo) to distal colon (DC) everted sacs from control-, DSS- and LPS-treated rats. (b) Ex vivo mannitol C^{14} transport from serosal to mucosal surfaces across ileum, proximal and the distal colon rat everted sacs. (c) Effect of verapamil (300 μM) on Rhodamine efflux in the ileum from water-treated or DSS-treated rats. Data are expressed as (%) rhodamine or mannitol C^{14} secreted/min/intestinal segment. Each column represents mean ± S.E.M. of $n = 6$ rats in each group. $^{**}P < 0.01$ versus control; $^{\#\#}P < 0.01$ vs. verapamil-treated.

Taken together, these results indicate that in DSS-induced colitis in rats, Pgp function is enhanced in the ileum and reduced in the proximal colon, which represent two sites characterized by no or very mild inflammation, respectively.

3.3. Kinetics of Pgp function in DSS-induced rat colitis

We next explored the time-dependant modification in rhodamine efflux in the proximal colon and in the ileum during DSS-induced colitis. In the proximal colon, the decrease in rhodamine efflux occurred as soon as day 3 after DSS (0.15 ± 0.022 versus 0.092 ± 0.017% rho 123 flux/min NS) and reached a maximal reduction at day 12 (0.043 ± 0.006% rho 123 flux/min) (Fig. 3a). In the Ileum, the rise of rhodamine efflux took place from day 3, and became maximal at day 7 (0.17 ± 0.021 versus 0.36, 0.06% rho 123 flux/min) (Fig. 3b).

3.4. Expression of Pgp in the ileum and the proximal colon of DSS-induced rat colitis

We analyzed Pgps expression in rat's intestinal tract using a monoclonal Pgp antibody. As shown on Fig. 4A, an immunoreactive band of 170 kDa was detected along the intestine with a maximal expression in the ileum and in the proximal colon. This profile of Pgp expression was in good agreement with Pgp activity found along the intestinal tract.

To determine whether the changes in Pgp function during inflammation were related to changes in the Pgp

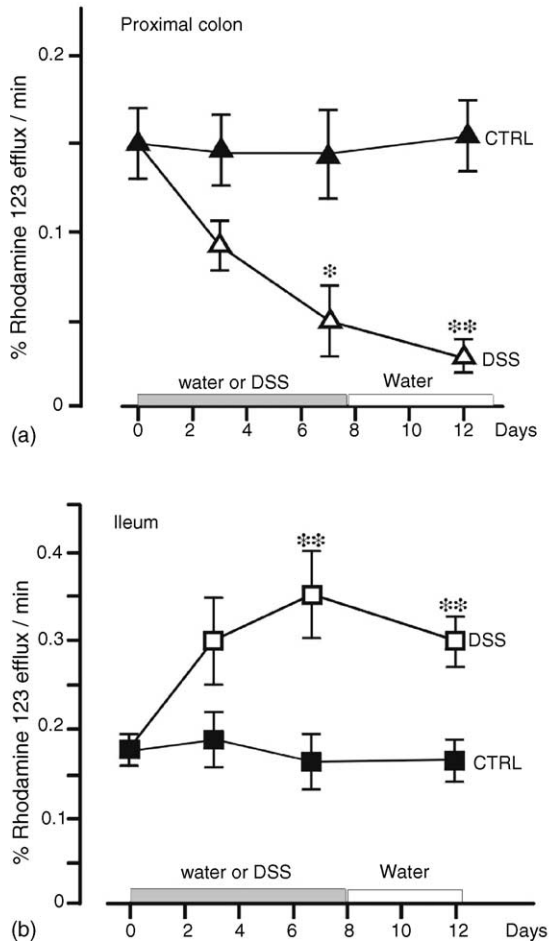


Fig. 3. Kinetic of Rhodamine efflux in intestine from control and DSS-treated animals. Ex vivo transport of rhodamine 123 from serosal to mucosal surfaces across rat everted intestinal sacs from the proximal colon (a) and the ileum (b) of DSS-treated rats and mice. Data are expressed as mean ± S.E.M. of (%) rhodamine secreted/min/intestinal segment from six animals per group. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. control.

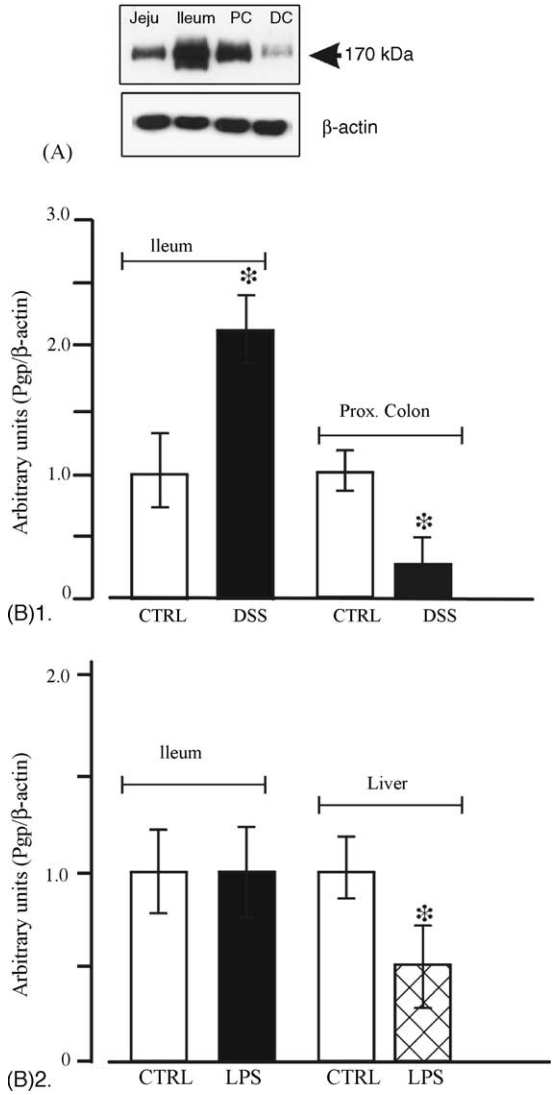


Fig. 4. (A) Pgp protein along the rat intestine. Western blot analysis of Pgp in 20 μg of crude membrane of rat jejunum, ileum, proximal and distal colon using the mouse monoclonal C219 antibody. Representative immunoblot of three separate experiments. One immunoreactive protein of 170 kDa was detected. (B) Abundance of Pgp protein during intestinal or systemic inflammation. Western blot analysis of Pgp in 20 μg of crude membrane prepared from the ileum and the proximal colon (PC) of control and DSS-treated rats (B1) or from the ileum and the liver of saline and LPS-treated rats (B2). Densitometric analysis of immunoblots was performed using the NIH image analysis (Scion Image corporation) and the results were normalized directly by β-actin expression. Data are expressed in arbitrary unit and are expressed as mean ± S.E.M. of $n = 6$ rats per group. $^{*}P < 0.05$ vs. control (CTRL).

protein levels, we performed a densitometry analysis of Pgp immunoblots. The immunoblots in question were normalized to β-actin expression, a constitutive enterocyte-specific protein whose level of expression appears to be relatively constant across individuals without any marked changes during DSS treatment. As shown on Fig. 4B1, the amount of Pgp protein was significantly increased (two-fold at day 12) in the ileum and decreased (two-fold) in the proximal colon of DSS-treated rats as compared to control rats.

Interestingly, LPS induced acute systemic inflammation did not significantly affect the levels of Pgp protein in the ileum. However, these levels of Pgp protein showed a two-fold decrease in the liver of LPS-treated rats as compared to saline-treated rats (Fig. 4B2), the result being in agreement with previous reports [13–15].

3.5. Intestinal Pgp function and expression are reduced in IL10$^{-/-}$ mice

Wild type and IL10$^{-/-}$ mice were sacrificed at the age of 4 months when all IL10$^{-/-}$ mice exhibited enterocolitis clinical signs such as diarrhea and melena, as previously described by Berg et al. [16]. In control mice (Fig. 5a), no significant difference in rho 123 efflux was observed along the intestinal tract (from duodenum to colon, with the colon being used entirely). However, IL10 deficient mice, with an inflammation affecting the small and large intestine, had a significant decrease in rhodamine efflux in each intestinal region (Fig. 5a). This decrease was three-fold in the duodenum ($P < 0.01$ versus control) and the jejunum ($P < 0.05$ versus control), and reached a 4-fold reduction

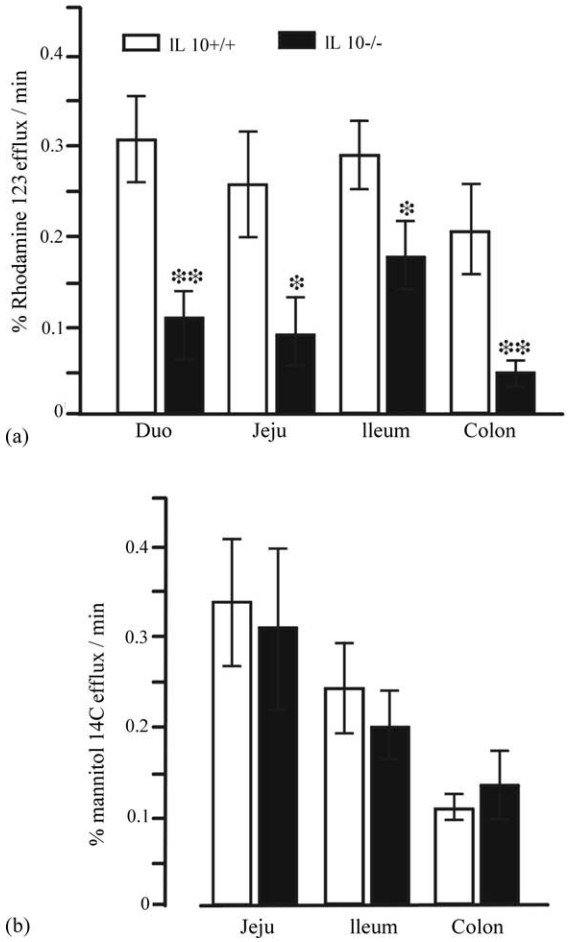


Fig. 5. Rhodamine (a) and Mannitol (b) transport in IL10$^{-/-}$ entrocolitis mice. Ex vivo transport of rhodamine 123 and Mannitol ^{14}C from serosal to mucosal surfaces across everted intestinal (duodenum, jejunum, ileum and the entire colon) sacs from IL 10$^{+/+}$ control mice and IL 10$^{-/-}$ mice. Data are expressed (%) rhodamine secreted/min/intestinal segment and are the means ± SEM of $n = 6$ mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control.

in the colon of IL10$^{-/-}$ mice ($P < 0.01$ versus control). These modifications in rhodamine efflux were not due to an increase in paracellular permeability since 14C mannitol transport was not modified in any of the intestinal regions studied (Fig. 5b).

This reduction in Pgp function was associated with a decrease in the Pgp protein levels. Using the same Pgp antibody as above, in control mice, we found that the amount of Pgp proteins is high in the small intestine and in the distal part of the colon with no or sparse expression in the proximal part of the colon (Fig. 6). IL10 deficient mice, exhibited a two- to four-fold decrease in Pgp expression in the duodenum, jejunum, ileum, and the distal colon as compared to wild type mice (Fig. 6).

4. Discussion

This study demonstrates for the first time that, IL10 deficient enterocolitis mice exhibit a decrease in Pgp function and expression all along the intestine as compared to control mice. Such an impaired function of Pgp occurs in the colon of DSS-induced colitis in rats. Unexpectedly, in this model the ileum which is not characterized by any signs of inflammation has a significant increase in Pgp function and expression. This finding suggests an intestinal adaptation process to luminal aggression which may have an impact on the pathogenesis of IBD.

Moreover, the expression of Pgp increases progressively from the proximal to the distal region of the small intestine, with an important individual variation, which is in agreement with previous observations reported in humans [3]. In rat's large intestine, Pgp expression decreases from proximal to distal colon consistent with a previous report [17]. This profile of Pgp expression in rat's colon contrasts with the relatively high expression in the distal colon reported in mice [18]. These results argue for a species specific difference in Pgp distribution along the intestinal tract and question the selection of animal models that surrogate human studies.

In this study, we show that IL10 deficient mice exhibit a reduction of intestinal epithelial Pgp activity all along the intestinal tract. It is well established that IL10 deficient mice bred under conventional conditions, spontaneously develop intestinal inflammation characterized by: discontinuous transmural lesions affecting the small and the large intestine and a dysregulated production of pro-inflammatory cytokines [16]. In these IL10$^{-/-}$ mice, the epithelial Pgp-mediated rhodamine 123 efflux decreases in every intestinal segment from the duodenum to the colon as compared to control, a result associated with a reduction in the levels of the membrane Pgp protein. These effects are likely to be mediated directly by the anti-inflammatory cytokine IL10 or indirectly through its repressive action on inflammatory cytokine production.

To our knowledge, very few studies, with conflicting results, have concerned the involvement of inflammatory mediators in the regulation of intestinal Pgp. In various intestinal cell lines, in vitro, pro-inflammatory cytokines (IL6, IFNγ, TNFα, IL2, and IL1β) can alternatively induce an increase or a decrease of Pgp according to the experimental model being used [19–22]. Therefore, the mediators released during intestinal inflammation, that could be putatively responsible for Pgps variation in the present in vivo study, remain to be identified.

In vivo, most studies dealing with the implication of inflammatory mediators in the regulation of Pgp have been addressed to the liver. Thus, induction of acute inflammation induced by LPS or turpentine in mice results in a down-regulation of hepatic levels of the Pgp protein and mRNA, that is mediated by IL-6 and IL-1β [13–15,23]. In this study, we confirm these results by showing that LPS-treated rats exhibit a reduction of hepatic Pgp expression and demonstrate that in these conditions, intestinal Pgp expression and function do not change in any of the intestinal segments. This indicates that intestinal Pgp expression and activity are sensitive to local and not or less to systemic inflammation.

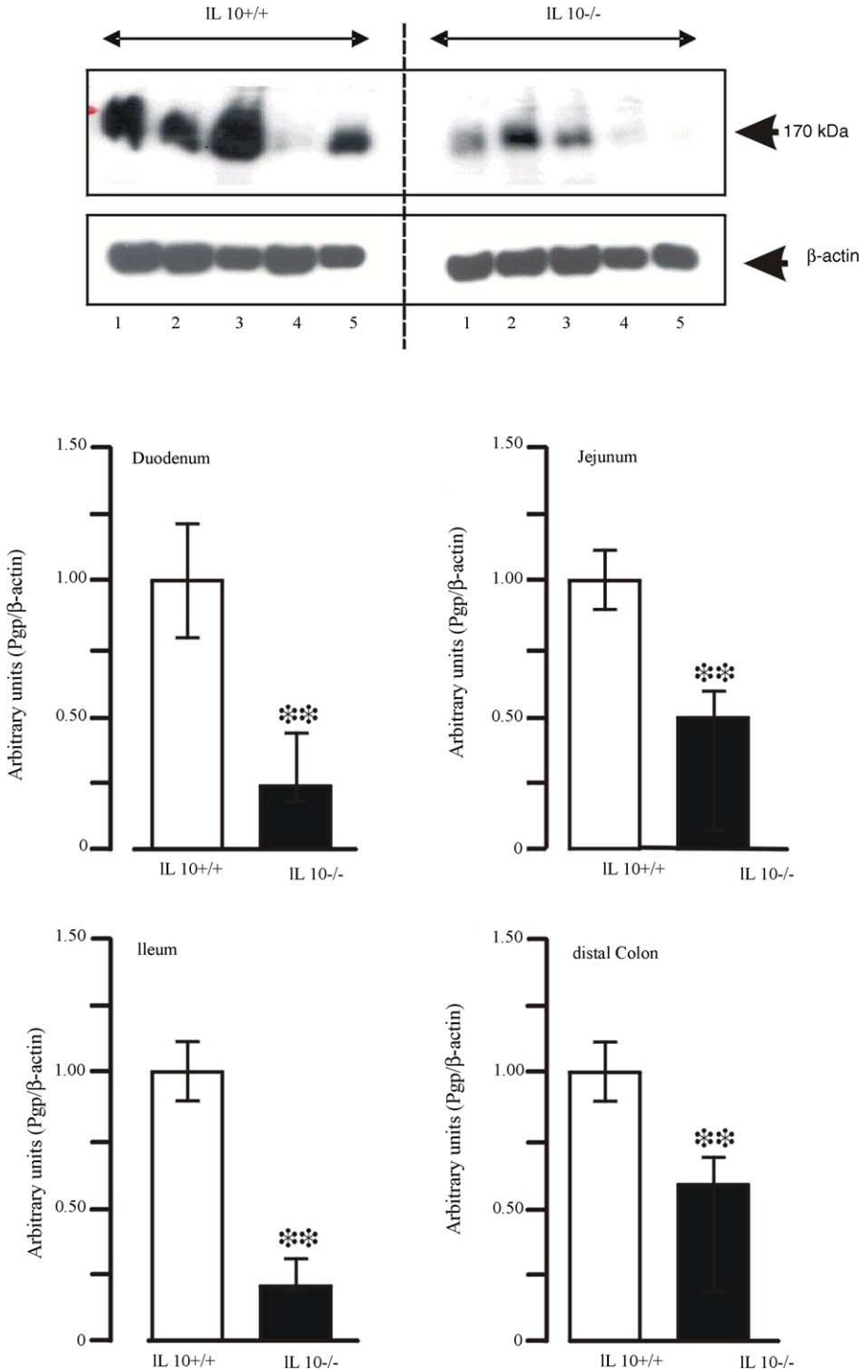


Fig. 6. Pgp protein along the intestine of mice. Western blot analysis of Pgp in 20 μg of crude membrane extracts from mice duodenum (lane 1), jejunum (lane 2), ileum (lane 3), proximal (lane 4) and distal colon (lane 5) from wild type IL10 ($IL10^{+/+}$) and IL10 deficient ($IL10^{-/-}$) mice, using the mouse monoclonal C219 antibody. One immunoreactive Pgp protein of 170 kDa was detected. Representative immunoblot of three separated experiments.Densitometric analysis of the Pgp protein normalized directly by β-actin expression using the NIH image analysis (Scion Image corporation). Data are expressed in arbitrary unit and are expressed as mean ± SEM of $n = 5$ mice per group. $^{**}P < 0.01$ vs. CTRL ($n = 5$).

This latter datum contrasts with the reported reduction of Pgp expression and the decrease in the basolateral >apical efflux of digoxin in jejunal segments of LPS-treated rats, mounted in Ussing chambers [24]. In that report, the presence and the degree of inflammation in the jejunum were assessed only by the increase of IL-6 mRNA expression as a marker of inflammation. Although we have no clear explanation, it would be interesting to elucidate these discrepancies taking into account the local intestinal inflammation of LPS-treated rats.

Interestingly, the impaired Pgp function in $IL10^{-/-}$ enterocolitis mice also occurs in DSS-induced rat colitis

in which inflammation is restricted to the large intestine as previously reported [7]. Our results are in agreement with a recent study reporting an early reduction of mdr1a expression in the colon of DSS-treated mice [25]. This down-regulation may correspond to a loss of apical epithelial cell's Pgp from the colonic mucosa during the active disease. However, whether Pgp expression is modified in other segments of the small intestine in DSS-induced rat colitis is unknown.

In this study, we provide evidence for such alteration by demonstrating an increase in the Pgp activity in the non-inflamed ileum but not, in the duodenum and the jejunum of DSS-treated rats. This effect is associated with an increase in the levels of the Pgp protein.

The enhancement of ileal Pgp function in DSS-induced rat colitis is likely to represent an adaptive mechanism that compensates the impaired activity of Pgp in the colon. The outcome of such an adaptive process would be to protect the mucosa against aggressive and harmful factors produced during inflammation. This is in accordance with the increased susceptibility of mdr1a$^{-/-}$ mice to develop a severe inflammation spread along the entire length of the colon when bred under specific pathogen-free conditions [4]. Indeed, the spontaneous development of colitis in mdr1a$^{-/-}$ mice [4] is the result of a mdr1a defect within the epithelial rather than within the lymphoid cell population or the intraepithelial lymphocytes (IEL) [26,27].

In human studies, it has been reported that Pgps expression and function in intraepithelial lymphocytes are lower in ulcerative colitis compared to Crohn's disease and healthy control [28]. On the other hand, peripheral blood lymphocytes from patients with UC and CD in whom medical therapy failed, have an increased expression of Pgp compared to inactive disease cases [29]. However, no significant difference in either IEL or intestinal epithelial cells of MDR expression between areas of active and inactive inflammation on colonic biopsies, was described.

In conclusion, these data are the first demonstration that IL10$^{-/-}$ enterocolitis mice exhibit an impaired function and expression of the epithelial cell's Pgp. They point out an adaptative process in the ileum occurring in DSS-induced colon histopathology in rodents and add new insights into the role of epithelial Pgp in the pathogenesis of IBD.

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