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Intestinal alkaline phosphatase contributes to the reduction of severe intestinal epithelial damage

Marianne Bol-Schoenmakers [a,*], Daniëlle Fiechter [a], Willem Raaben [b], Ine Hassing [a], Rob Bleumink [a], Daniëlle Kruijswijk [b], Kelly Maijoor [b], Monique Tersteeg-Zijderveld [c], Ruud Brands [a], Raymond Pieters [a]

[a] Institute for Risk Assessment Sciences, Department of Immunotoxicology Utrecht University, Utrecht, The Netherlands
[b] AM Pharma BV, Bunnik, The Netherlands
[c] Institute for Risk Assessment Sciences, Department of VPH, Utrecht University, Utrecht, The Netherlands

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ABSTRACT

Inflammatory bowel disease is characterized by chronic inflammation of the intestine and is accompanied by damage of the epithelial lining and by undesired immune responses towards enteric bacteria. It has been demonstrated that intestinal alkaline phosphatase (iAP) protects against the induction of inflammation, possibly due to dephosphorylation of lipopolysaccharide (LPS). The present study investigated the therapeutic potential of iAP in intestinal inflammation and epithelial damage. Intestinal epithelial damage was induced in C57BL/6 mice using detran sulfate sodium (DSS) and iAP was administered 4 days after initial DSS exposure. Loss in body weight was significantly less in iAP-treated mice and accompanied with reduced colon damage (determined by combination of crypt loss, loss of goblet cells, oedema and infiltrations of neutrophils). Treatment with iAP was more effective in case of severe inflammation compared to situations of mild to moderate inflammation. Rectal administration of LPS into a moderate inflamed colon did not aggravate inflammation. Furthermore, soluble iAP did not lower LPS-induced nuclear factor-κB activation in epithelial cells in vitro but induction of cellular AP expression by butyrate resulted in decreased LPS response. In conclusion, the present study shows that oral iAP administration has beneficial effects in situations of severe intestinal epithelial damage, whereas in moderate inflammation endogenous iAP may be sufficient to counteract disease-aggravating effects of LPS. An approach including iAP treatment holds a therapeutic promise in case of severe inflammatory bowel disease.

1. Introduction

Inflammatory bowel diseases, including Crohn's disease and ulcerative colitis, are multifactorial diseases characterized by an excessive intestinal inflammation and disruption of the intestinal epithelial lining. Evidence is growing that activation of mucosal immune responses by commensal enteric bacteria is an important factor in the etiology of inflammatory bowel disease (Rath et al., 2001; Rhee et al., 2005; Sartor, 2004). In line with this, certain genetic polymorphisms related to microbial responses, including those of Toll-like receptor (TLR)4, have been associated to susceptibility to inflammatory bowel disease (Gribar et al., 2008). Conceivably, lipopolysaccharide (LPS) which binds to TLR4 may be involved in aggravating inflammatory bowel disease. Methods to detoxify LPS may therefore be an interesting therapeutic venue in inflammatory bowel disease. Interestingly, it has been shown that the enzyme alkaline phosphatase (AP) can detoxify LPS by dephosphorylation of the lipid A moiety (Poelstra et al., 1997a; Poelstra et al., 1997b).

AP is a naturally occurring enzyme with ubiquitous distribution among cell types and tissues. The intestinal isoform of AP, intestinal AP (iAP) (Sanchez de Medina et al., 2004), is expressed in high amounts in the intestinal lining (Goldberg et al., 2008; Tuin et al., 2009) and is considered a differentiation marker of intestinal epithelial cells (IEC) (Fukushima et al., 1998; Guzman-Aranguez et al., 2005; Lee et al., 2005). iAP expression by differentiated IEC is inhibited in patients suffering from ulcerative colitis, possibly by pro-inflammatory cytokines like tumor necrosis factor (TNF)-α which are abundantly present in an inflamed tissue (Malo et al., 2006; Tuin et al., 2009). iAP appears essential in controlling responses towards the intestinal flora and maintaining epithelial integrity, and loss of iAP expression increases susceptibility to inflammation and sepsis (Bates et al., 2007; Geddes and Philpott, 2008; Goldberg et al., 2008).

Previous studies have demonstrated that in addition to homeostatic and disease-protective effects iAP administration may also have therapeutic effects in several LPS-mediated diseases, including sepsis and lung toxicity (Bentala et al., 2002; Beumer et al., 2003; Koyama et al., 2004; Verweij et al., 2004). Recently, it has been demonstrated

* Corresponding author. Utrecht University, IRAS, IT P.O. Box 80177 NL 3508 TD Utrecht, The Netherlands. Tel.: +31 30 2535326; fax: +31 30 2535077.
E-mail address: m.bol-schoenmakers@uu.nl (M. Bol-Schoenmakers).

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that administration of AP protects against the induction of dextran sulphate sodium (DSS)-mediated intestinal epithelial damage and inflammation in rats, when given prior to the DSS insult (Tuin et al., 2009). In the present study, we investigated the effect of administration of iAP in mice after disruption of intestinal epithelial lining by DSS. Results show beneficial effects of iAP administration, but only in cases of severe intestinal injury and inflammation. Additional in vitro data suggest that in mild cases of epithelial damage, endogenous AP may suffice to protect against further disease aggravation by LPS.

2. Methods

2.1. Mice and reagents

Twelve-week-old female specific pathogen-free C57BL/6J mice purchased from Charles River (France) were maintained under barrier conditions in filter-topped macrolon cages with wood-chip bedding, at a mean temperature of 23 ± 2 °C, 50–55% relative humidity, and 12 h light/dark cycle. Acidified drinking water and laboratory food pellets were provided *ad* libitum. The experiments were approved by the Animal Experiments Committee of the Utrecht University.

Clinical grade bovine intestinal AP (iAP) from Biozyme (Blaenavon, United Kingdom) was obtained through AM-Pharma (Bunnik, The Netherlands). Other chemicals were obtained from Sigma Aldrich (Zwijndrecht, The Netherlands), unless stated otherwise.

2.2. Induction of intestinal epithelial damage and treatment with iAP and LPS

Mice were divided in treatment group of 5 to 10 animals and body weight, stool consistency and behaviour were recorded daily during the experiment. To induce acute intestinal epithelial damage, C57BL/6 mice were provided with drinking water containing 2% (w/v) DSS (MP Biomedicals, Aurora, OH; MW 35–50 kD) for 5 consecutive days, followed by normal drinking water until they were sacrificed (day 12). No differences in water consumption between treatment groups were observed.

Oral treatment with iAP started 4 days after start of DSS exposure and was continued until mice were sacrificed. Treatment was performed by daily intra-gastric gavage of 100 U of iAP in Tris–HCl buffer, pH 7.2 in a 100 µl bolus. Vehicle-treated animals received 100 µl of Tris–HCl buffer alone.

In case of LPS exposure mice received 100 µl LPS (O111:B4, Sigma; 100 µg as low dose or 500 µg as high dose) dissolved in PBS via rectal enema under isofluran/O_2 anaesthesia four days after initial DSS exposure and dosing was repeated every other day (4 times in total).

2.3. Evaluation of intestinal inflammation

After mice were sacrificed the colon was dissected from anus to caecum. The colon length was recorded and subsequently the faeces were carefully removed. Colon was weighed and then cut longitudinally, and the full length of half of the colon was fixed in phosphate-buffered formalin and embedded in paraffin. Routine 5 µm sections were prepared and stained with haematoxylin-eosin and evaluated in a blind coded fashion by two independent investigators (MBS and RB).

Histological structural alterations were assessed by considering following parameters scored on a 0–3 scale (0 = no alterations; 1 = mild mucosal alterations in less than 1/3 of the colon; 2 = moderate mucosal and submucosal alterations involving up to half of the colon; and 3 = severe alterations in more than half of the colon): oedema; crypt loss; loss of goblet cells and leukocyte infiltrations. The histological score was calculated as the sum of the scores for each category and resulted in a maximum score of 12 in the most severe situation. Evaluation of independent investigators was averaged and resulted in a histological score for each mouse.

2.4. Determination of disease grade

Disease severity in individual animals was ranked from 0 to 4 based on maximum body weight loss, effect on colon length and colon weight, histological score, myeloperoxidase activity and TNFα release from distal colon samples (Table 1). Scores per parameter were averaged resulting in a disease grade for every individual mouse.

2.5. Myeloperoxidase activity

A part of the dissected colon of 20 to 40 mg (exact weight was recorded) was snap-frozen in liquid nitrogen and stored at −20 °C. Tissue was thawed and homogenized by means of a Braun Potter in 500 µl of ice-cold Tris–HCl lysis buffer (15 mM), pH 7.4, containing 300 mM NaCl, 2 mM $MgCl_2$, 1% triton X100 and 1 µg/ml of the protease inhibitors leupeptin A and aprotinine. Homogenate was centrifuged for 7 min at 700 × *g*, supernatant was taken and centrifuged for another 10 min. at 12000 × *g* after which 10 µl of the supernatant was diluted 5 times in 0.5% hexadecyltrimethylammonium bromide (HETAB) in 50 mM potassium phosphate buffer pH 6.0. Myeloperoxidase activity was determined by adding 150 µl ready to use 3,3′,5,5′-tetramethylbenzidine substrate and increase in absorbance at 630 nm was recorded for at least 15 min. One unit of myeloperoxidase activity was defined as the amount of enzyme causing an increase in absorbance of 0.001 per 5 min. The result was normalized for colon tissue weight.

2.6. Colon culture and cytokine analysis

A distal sample of the dissected colon of 30 to 50 mg (exact weight was recorded), was washed three times in ice-cold PBS, containing 40 mg/ml glucose, 100 IU/ml penicillin–streptomycin and 250 µg/ml fungizone and incubated in 0.5 ml complete RPMI1640 (Invitrogen Life Technologies, Paisley, Scotland) supplemented with 10% heat-inactivated fetal calf serum (Greiner Bio-one, Germany) for 24 h at 37 °C, 5% CO_2. Supernatant was collected and stored at −20 °C until analysis was performed.

Interleukin (IL)-12 and IL-1β were determined by sandwich ELISA. IL-12 capture and biotin antibodies were obtained from BD Pharmingen (Erembodegem, Belgium), IL-1β antibodies were from R&D (Minneapolis, MN). Highbond plates (Costar 3590) were coated

Table 1
Ranking of disease parameters.

Grade	Maximum body weight loss (%)	Colon length (cm)	Relative colon weight (mg/g BW)	Colon morphology (hist. score)	TNF-α release (ng /g colon)	MPO activity (*10^3 U/g colon)
0	<2	>8	<8.5	<1	<1.5	<1.5
1	2–5	7–8	8.5–10	1–3	1.5–3	1.5–3
2	5–10	6.5–7	10–12	3–6	3–6	3–6
3	10–15	6–6.5	12–14	6–9	6–10	6–10
4	>15	<6	>14	>9	>10	>10

overnight at 4 °C with 2 μg/ml rat anti-mouse IL-1β or rat anti-mouse IL-12 p40/p70 in PBS, washed and blocked with PBS-Tween 20/1% bovine serum albumin (PBS-T/BSA) for 4 h at room temperature. Samples, IL-1β and IL-12 standards were added in several dilutions and incubated overnight at 4 °C. After washing, plates were incubated with 0.25 μg/ml goat anti-mouse IL-1β conjugate or 0.5 μg/ml rat anti-mouse IL-12 conjugate diluted in PBS–T/BSA for 1 h at room temperature. Plates were washed and incubated with streptavidin-horse radish peroxidase (0.1 μg/ml, Sanquin, Amsterdam, The Netherlands) diluted in PBS-T/BSA for 45 min at room temperature. After the final washes, 3,3′,5,5′-tetramethylbenzidine substrate (0.1 mg/ml) was added and the color reaction was stopped after 15 minutes with 2 M H_2SO_4. Absorbance was measured at 450 nm. IL-10 (BD Pharmingen) and TNF-α ELISA (Biosource, Nivelles, Belgium) were performed according to the instructions of the manufacturer. Cytokine levels were normalized for weight of colon tissue used in culture.

2.7. Cell culture

The murine small intestinal cell line m-ICc12, stably transfected with a nuclear factor-κB (NF-κB) luciferase reporter construct, was a kind gift from Dr. A. Vandewalle (INSERM, Paris, France (Bens et al., 1996; Hornef et al., 2003)). m-ICc12 cells were cultured in DMEM/F12 (1:1 v/v), supplemented with 100 IU/ml penicillin/streptomycin, 60 nM selenium, 5 g/ml bovine transferrine, 2 mM glutamine, 5 g/ml bovine insulin, 2 g/l glucose, 50 nM dexamethasone, 1 nM triiodothyronine, 10 ng/ml epidermal growth factor, 2% fetal calf serum, 50 μg/ml hygromycin and 15 mM HEPES. Cells were grown in a humidified incubator at 37 °C and 5% CO_2.

2.8. AP activity

m-ICc12 cells were grown to confluence in 75 cm^2 flask and incubated with sodium butyrate for 48 h. To quantify cell numbers, images were taken at 6 different spots by means of an inverted microscope (Olympus CKX41 with a DP-20 camera) and cells were counted using imaging software (Olympus). Cells were rinsed tree times with 0.9% NaCl and harvested in 2.5 mM glycine buffer (pH = 9.6) with a rubber policeman. Cell lysates were mixed with 2.4 mg/ml p-nitrophenylphosphate in glycine buffer, supplemented with 8.5 mM MgCl2, and an increase in absorbance at 405 nm was recorded for 1 h. One unit of AP activity was defined as the amount of enzyme causing the hydrolysis of 1 μM of p-nitrophenylphosphate per min (pH = 9.6; 25 °C). The result was normalized for the amount of cells.

2.9. NF-κB activation and mouse inflammatory protein-2 (MIP-2) release

m-ICc12 cells were grown to confluence in 96-wells plates (Greiner Bio-one, Germany) and subsequently incubated with

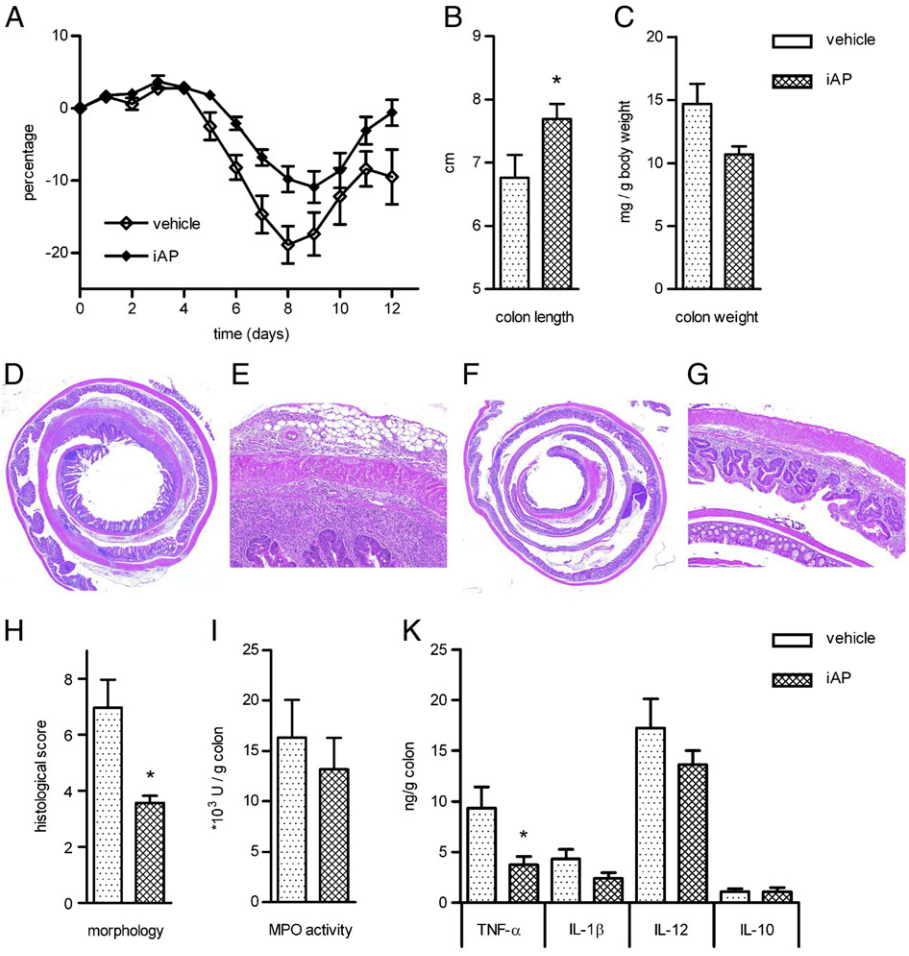

Fig. 1. Treatment with iAP after DSS-induced intestinal damage.DSS was administered via drinking water during 5 days, followed by normal drinking water. iAP was administered daily from day 4 and continued until mice were sacrificed. B–J: data obtained on day 12; group size: $n = 6$ (vehicle) and $n = 10$ (iAP) A. body weight changes during experimental procedure. B. Colon length. C. Relative colon weight. D–G: H–E stained paraffin sections from vehicle-treated (D–E) or iAP-treated (F–G) colon. Magnitude ×40 (D; F) and ×200 (E; G). H. changes in colon morphology, scored as described in material and methods I. myeloperoxidase activity in colon homogenate. K. cytokine levels in supernatant of cultured distal colon sample. Data are represented as mean ± S.E.M.. * $p < 0.05$ significantly different from vehicle-treated group.

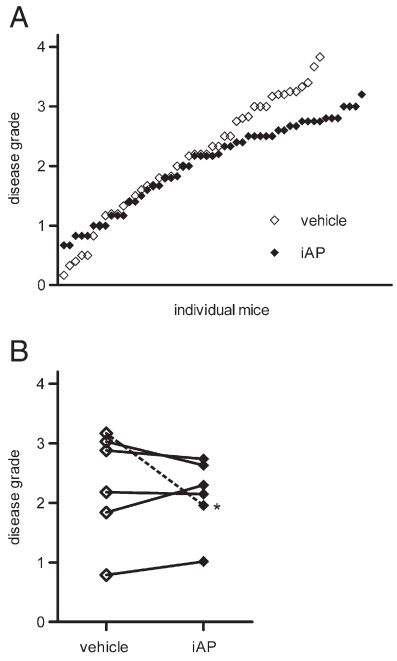

Fig. 2. iAP effect in relation to severity of intestinal inflammation.Disease grading is based on maximum body weight loss, colon length and weight, histological score, TNFα release and myeloperoxidase activity and calculated as indicated in Table 1. A. Each dot represents one single animal, open symbols: vehicle treatment, closed symbols: iAP treatment. B. Each line represents one separate study. Symbols represent average disease grade per treatment group ($n=6$ to 10) within one study. The dotted line represents the study shown in detail in Fig. 1. *: $p=0.0046$ (difference between iAP treatment and vehicle treatment).

different amounts of iAP for 30 min, or with sodium butyrate for 48 h. LPS (0.1–10 ng/ml; O111:B4, Sigma) or mouse IL-1β (0.1–1 ng/ml; e-bioscience, San Diego, USA) was added, and cells were cultured for another 4 h. Culture supernatant was harvested and stored at −20 °C until determination of MIP-2. Cells were washed once with PBS, and lysed with 20 µl lysis buffer (Promega, Madison WI, USA). Luciferase activity was recorded by means of a luminometer (LUMIstar OPTIMA, BMG Labtech) using a luciferase assay kit (BioThema, Sweden). MIP-2 ELISA (KOMA Biotech., Seoul, Korea) was performed according the instructions of the manufacturer.

2.10. Statistics

Data from animal studies are presented as the group mean ± standard error of the mean (S.E.M.) and analyzed using SigmaStat statistical software package (SPSS, Chicago, IL). A Kruskal–Wallis test was performed for all groups together. When appropriate, Mann–Whitney tests were performed to determine differences between two groups. Differences were considered significant when p values were <0.05. Data from in vitro tests are presented as mean ± standard deviation (S.D.) and analyzed using GraphPad Prism software. Two-way ANOVA was performed followed by Bonferroni post-hoc test. Differences were considered significant when p values were <0.05.

3. Results

3.1. iAP treatment ameliorated DSS-induced intestinal epithelial damage

Previously, it has been demonstrated that iAP can reduce the severity of DSS-induced inflammation in rats. In that particular study iAP was administered during induction of intestinal epithelial damage (Tuin et al., 2009). To investigate the therapeutic effect of iAP in intestinal inflammation, C57BL/6 mice were first exposed to DSS for 5 days, and treated with iAP from day 4 onward. Body weight loss was observed from day 4 and reached maximum at day 8 (Fig. 1A). Observed changes in stool consistency (loose stool or even absence of stool from day 5 to 12; data not shown) confirmed the induction of colon inflammation. No mice died or had to be sacrificed as a result of

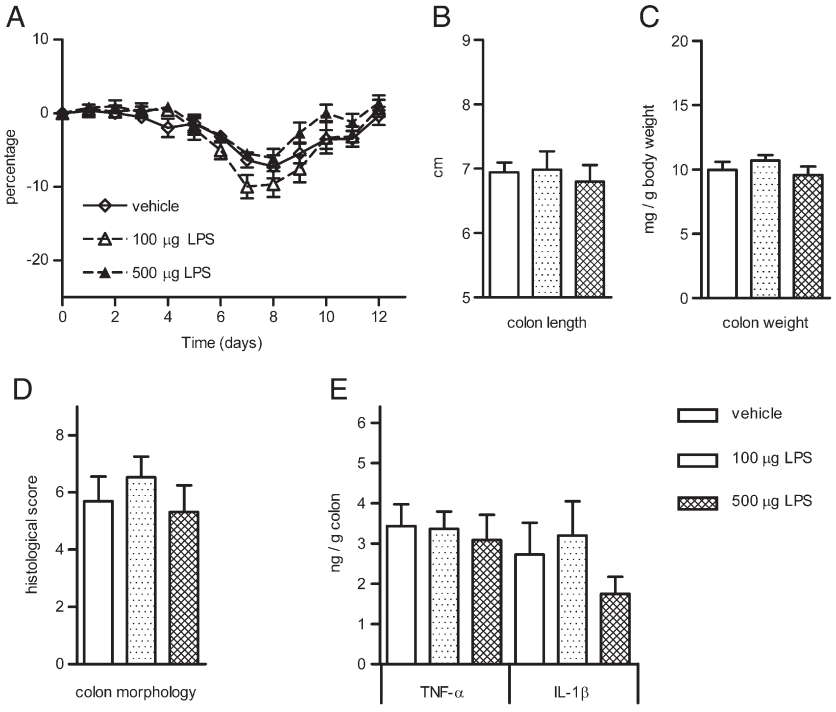

Fig. 3. Effect of rectal LPS administration on intestinal inflammation.DSS was administered via drinking water during 5 days, followed by normal drinking water. LPS was administered every other day from day 4 and continued until mice were sacrificed. B–E: data obtained on day 12, group size $n=9$ (vehicle and 100 µg/ml LPS), $n=7$ (500 µg/ml LPS). A. body weight changes during experimental procedure. B. Colon length. C. Relative colon weight. D. Changes in colon morphology, scored as described in material and methods. E. Cytokine in supernatant of cultured distal colon sample. Data are represented as mean ± S.E.M.

DSS exposure. Significant reduction of colon length (Fig. 1B) accompanied with markedly increased colon weight (Fig. 1C) was observed in DSS-exposed mice in comparison to mice that received normal drinking water (healthy subjects: colon length 8.2 ± 0.4 cm; relative colon weight 8.6 ± 0.7 mg/g bodyweight, data not shown). These data were indicative of colon inflammation.

Oral administration of iAP was found to diminish DSS-induced body weight loss and effects on colon length and weight (Fig. 1B–C). In addition, histological scoring (Fig. 1D–H) confirmed the beneficial effect of iAP administration although myeloperoxidase activity in colon homogenates (Fig. 1I), serving as a marker for activity of neutrophils, was not reduced. Of the cytokines evaluated only TNF-α was significantly reduced (Fig. 1K). Importantly, water intake was not different between vehicle-treated and iAP-treated groups (data not shown), indicating that the exposure to DSS was comparable between groups.

3.2. Beneficial effects of iAP were more pronounced in situations of severe inflammation.

In the described experiment DSS exposure resulted in severe intestinal epithelial damage and a clear effect of iAP treatment was found on colon inflammation. The experiment was repeated several times and overall results indicated that the beneficial effects of iAP were most obvious in cases with severe epithelial damage. To assess the influence of disease severity we calculated a disease grade for every individual animal (95 in total used in 6 experiments and of which 44 were vehicle-treated and 51 were iAP-treated) based on maximum body weight loss, colon length and weight, histological changes, myeloperoxidase activity and TNF-α release in the colon. By ranking disease severity in this way effects on both clinical appearance and colon inflammation are taken into account. In the different experiments performed individual mice clearly displayed a wide range of disease severity after DSS exposure (Fig. 2), but more importantly, it is apparent that mice treated with iAP do not reach disease scores as high as vehicle-treated mice, confirming that iAP is most potent in case of severe inflammation. Also when disease scores were evaluated per experiment the beneficial effects of iAP were most distinct in those experiments in which intestinal epithelial damage was most severe (Fig. 2B).

3.3. Rectal LPS administration did not aggravate DSS-induced intestinal epithelial inflammation.

In systemic diseases such as sepsis the beneficial effect of iAP was attributed to its capacity to detoxify LPS (Poelstra et al., 1997b). To investigate the direct influence of LPS on DSS-induced intestinal epithelial damage, LPS was administered rectally to mice which were already exposed to DSS for four days. DSS exposure was continued for 1 day, after which animals received normal drinking water. Mice received 100 μg or 500 μg LPS via a rectal enema 4 times every other day starting on day 4 of DSS exposure. These doses are in the range of the total amount of LPS present within the lumen in case of gram-negative bacterial infection (Rogers et al., 1985). The severity of disease induced in this experiment was moderate (disease score of 1.90 ± 0.12). As shown in Fig. 3A, LPS administration did not significantly alter DSS-induced body weight loss. In addition, no effects were found on colon length, weight or on morphology of the colon after LPS administration (Fig. 3B–D). Furthermore, LPS treatment did not significantly alter TNF-α and IL-1β release from distal colon samples (Fig. 3E). Data shows that local LPS administration does not substantially modulate a moderate colonic inflammation.

3.4. iAP had no effect on LPS-induced NF-κB activation and MIP-2 release in intestinal epithelial cells.

Next, we investigated whether iAP could directly modulate LPS-induced epithelial cell responses. Mouse intestinal epithelial cells (m-ICc12), stably transfected with a NF-κB-luciferase reporter construct, were incubated with iAP for 30 min, after which various amounts of LPS and IL-1β were added. NF-κB activation and MIP-2 release were analyzed after 4 h of stimulation. Both LPS and IL-1β were able to stimulate m-ICc12 cells to activate NF-κB and to release MIP-2. iAP was not able to decrease LPS- or IL-1β-induced NF-κB activation (Fig. 4A–B) or MIP-2 release (Fig. 4C).

3.5. Culturing intestinal epithelial cells in the presence of butyrate resulted in inhibition of LPS-induced NF-κB activation.

Addition of sodium butyrate to intestinal epithelial cells has been shown to induce cell differentiation accompanied by up-regulation

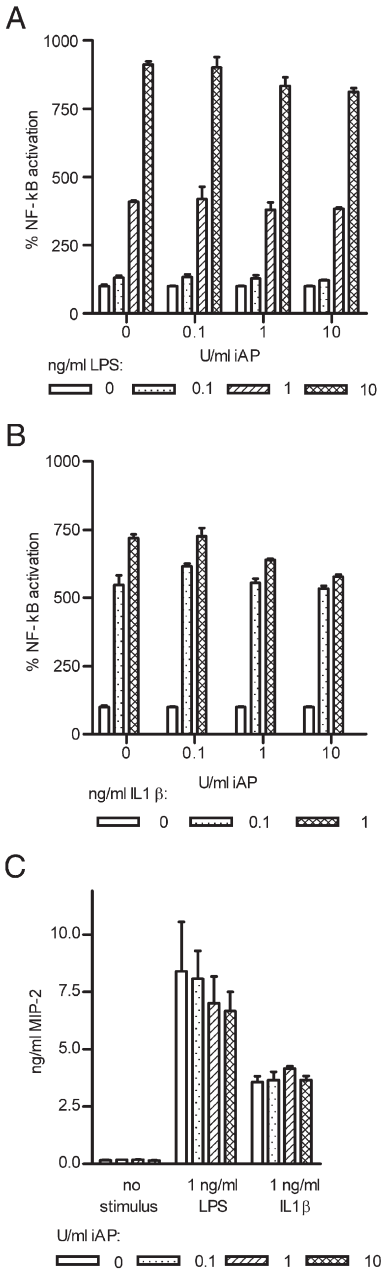

Fig. 4. Effect of iAP addition on NF-κB activation and MIP-2 release in m-ICc12 cells in vitro. m-ICc12 cells were cultured to confluence, incubated with indicated doses of iAP for 30 min, and subsequently stimulated with LPS or IL-1β for 4 h. A–B: NF-κB activation is measured using a luciferase reporter construct and expressed as percentage of control (no stimulus). C. MIP-2 is measured in culture supernatant using ELISA. Data are presented as mean ± S.D. from a triplicate from one representative experiment out of three.

of intracellular AP activity in several intestinal epithelial cell-lines (Fukushima et al., 1998) Therefore, we studied the effect of sodium butyrate on LPS-induced NF-κB activation by m-ICc12 cells. Addition of sodium butyrate to m-ICc12 cells resulted in immediate inhibition of cell proliferation and induced morphological changes in the cultured cells (Fig. 5A) and an increase in AP activity (Fig. 5B). In the presence of sodium butyrate less NF-κB activation was induced by LPS (Fig. 5C). In contrast, sodium butyrate did not affect the response of m-ICc12 cells to IL-1β thus excluding a direct effect on NF-κB activation (Fig. 5D). The results indicate that butyrate specifically inhibits LPS-mediated NF-κB activation in m-ICc12 cells, and also induces up-regulation of AP activity and cell differentiation.

4. Discussion

Support is growing that host inflammatory responses to commensal bacteria and their products contribute to inflammatory bowel disease (Sartor, 2004). Therefore, modification of pro-inflammatory microbial molecules may add to the treatment of inflammatory bowel disease.

In the present study, we show that oral treatment with iAP is of benefit to mice with existing (DSS-mediated) intestinal inflammation and damaged intestinal epithelial lining. This extends knowledge on the therapeutic potential of iAP preventing DSS-mediated intestinal inflammation in rats (Tuin et al., 2009). The mode of action by which iAP may have its therapeutic effect is via the dephosphorylation and hence detoxification of luminal LPS (Koyama et al., 2002; Poelstra et al., 1997b). Unfortunately, direct effects of iAP on LPS dephosphorylation in vivo could not be assessed, since the Limulus amoebocyte lysate (LAL) assay cannot discriminate between LPS and dephosphorylated LPS (Takayama et al., 1984).

Intriguingly, our data suggest that iAP is only therapeutically effective when the inflammation exceeds a certain disease grade. In those severe cases, colon inflammation and body weight loss were less pronounced and body weight recovered more quickly. Addition of high amounts of LPS directly into the lumen of injured colon did not aggravate a moderate DSS-induced colonic injury.

Our in vivo findings, together with the presented in vitro data may explain why administration of iAP is only beneficial in cases of severe intestinal damage. Notably, our in vitro data demonstrate that addition of iAP to intestinal epithelial m-ICc12 cells did not inhibit LPS-induced NF-κB activation, but that culturing m-ICc12 epithelial cells in the presence of butyrate, known to upregulate iAP (Fukushima et al., 1998; Guzman-Aranguez et al., 2005; Lee et al., 2005), inhibited the NF-κB-response to LPS but not to IL-1β. So, endogenously produced iAP may protect against LPS-induced cellular responses. These findings are in line with a recent study showing that LPS-induced phosphorylation of IκB-α and RelA/p65 and activation of NF-κB is reduced in AP-transfected intestinal HT29 cells that produce the enzyme endogenously (Goldberg et al., 2008). In addition, a recent study in zebrafish demonstrated a protective effect of iAP expression against LPS-mediated inflammation (Bates et al., 2007).

In general, differentiated epithelial cells produce high amounts of iAP. Our data suggest that in cases of moderate intestinal epithelial injury, cell-bound iAP may exert sufficient modulating capacity, that administration of iAP is not of additional benefit and that addition of LPS is unable to increase disease severity. However, in case of severe inflammation with profound epithelial damage, loss of differentiated cells results in reduced iAP expression, and addition of exogenous iAP will have a therapeutic effect. This hypothesis fits with findings by Tuin et al (Tuin et al., 2009) demonstrating a reduced AP expression in colon biopsies of patients with ulcerative colitis and Crohn's disease and in rats with DSS-induced intestinal epithelial damage, although a comparison between the efficacy of iAP and the severity of the disease was not made.

The anti-inflammatory capacity of butyrate is well-documented both in vitro and in vivo (Malo et al., 2006; Tedelind et al., 2007; Weng et al., 2007), and several clinical studies have demonstrated beneficial effects of butyrate in inflammatory bowel disease (Di Sabatino et al.,

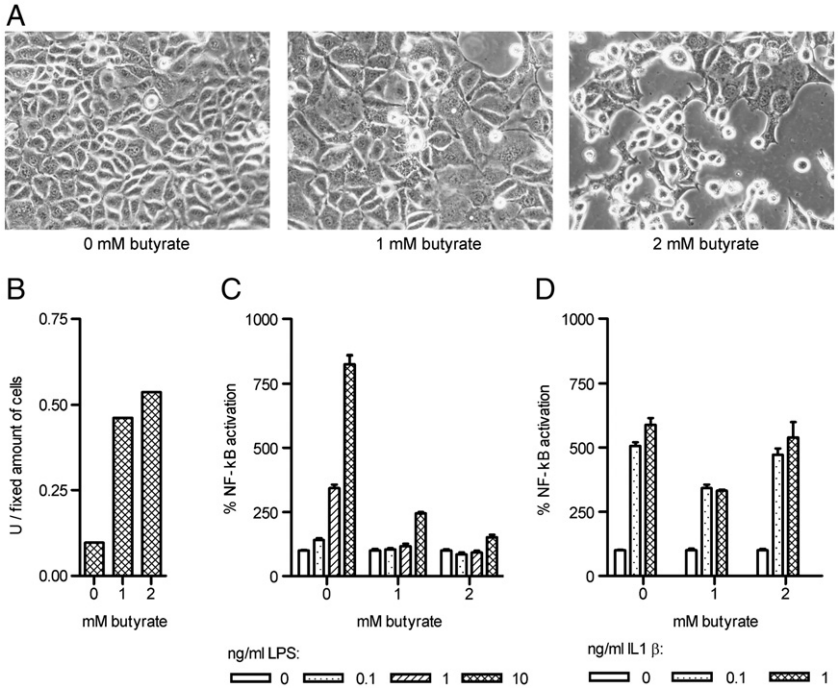

Fig. 5. Effect of butyrate on LPS-induced NF-κB activation of m-ICc12 cells.m-ICc12 cells were cultured to confluence and incubated with indicated amounts of sodium butyrate for 48 h. A. phase images of cells after 48 h culture with indicated concentrations of sodium butyrate, magnitude ×200 B. AP activity is expressed as arbitrary units per fixed amount of cells. C–D. Cells were subsequently stimulated with LPS or IL-1β for 4 h. NF-κB activation is measured using a luciferase reporter construct and expressed as percentage of control (no stimulus). Data are presented as mean ± S.D. from a triplicate from one representative experiment out of three.

2005; Steinhart et al., 1996). The data shown here indicate that the relieve of colitis by butyrate may be at least partly attributable to the induction of cellular expression of iAP resulting in interference with LPS-mediated effects. This possibility adds to other mechanisms as recently described (Maslowski et al., 2009).

The involvement of LPS in intestinal inflammation is in line with data showing immune responses against the own intestinal flora in patients suffering from inflammatory bowel disease (Rath et al., 2001). It has been shown that LPS inhibits epithelial restitution, resulting in aggravation of intestinal inflammation (Qureshi et al., 2005). Treatment with antibiotics is therefore often of benefit (Sartor, 2004). On the other hand, Fukata et al. (2006) demonstrated that epithelial repair in the intestine depended on TLR4 signaling, hence on the presence of LPS. In fact, activation of TLRs by commensal bacteria plays an essential role in maintaining gut homeostasis (Lee et al., 2007; Rakoff-Nahoum et al., 2004). Of interest is the finding by Bates et al that the expression of AP in intestinal epithelium of zebrafish also depended on TLR signaling (Bates et al., 2007). This implicates that LPS in itself induces a process to prevent extensive TLR4 signaling. The multiple actions of LPS complicate the interpretation of its in vivo effects in inflamed intestines.

Together, findings in the present study add to the growing evidence that iAP is of importance in maintaining gut homeostasis (Geddes and Philpott, 2008), and importantly demonstrates that iAP not only prevents intestinal inflammation but may indeed also have therapeutic effects, in particular in cases of severe colon inflammation. AP may thus be considered in treatment strategies of patients with severe colitis.

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