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Intestinal Alkaline Phosphatase Has Beneficial Effects in Mouse Models of Chronic Colitis

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Abstract

Background—The brush border enzyme intestinal alkaline phosphatase (IAP) functions as a gut mucosal defense factor and is protective against dextran sulfate sodium (DSS)-induced acute injury in rats. The present study evaluated the potential therapeutic role for orally administered calf IAP (cIAP) in two independent mouse models of chronic colitis: (1) DSS-induced chronic colitis, and (2) chronic spontaneous colitis in Wiskott-Aldrich Syndrome protein (WASP) deficient (knockout) mice that is accelerated by irradiation.

Methods—The wild-type (WT) and IAP knockout (IAP-KO) mice received 4 cycles of 2% DSS *ad libitum* for 7 days. Each cycle was followed by a 7-day DSS-free interval during which mice received either cIAP or vehicle in the drinking water. The WASP-KO mice received either vehicle or cIAP for 6 weeks beginning on the day of irradiation.

Results—Microscopic colitis scores of DSS-treated IAP-KO mice were higher than DSS-treated WT mice (52 ± 3.8 vs. 28.8 ± 6.6 , respectively, $P < 0.0001$). cIAP treatment attenuated the disease

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Competing interests:

The authors declare no conflicting financial interests.

in both groups ($KO = 30.7 \pm 6.01$, $WT = 18.7 \pm 5.0$, $P < 0.05$). In irradiated WASP-KO mice cIAP also attenuated colitis compared to control groups (3.3 ± 0.52 vs. 6.2 ± 0.34 , respectively, $P < 0.001$). Tissue myeloperoxidase activity and pro-inflammatory cytokines were significantly decreased by cIAP treatment.

Conclusions—Endogenous IAP appears to play a role in protecting the host against chronic colitis. Orally administered cIAP exerts a protective effect in two independent mouse models of chronic colitis and may represent a novel therapy for human IBD.

Keywords

DSS-induced chronic colitis; WASP-KO and spontaneous chronic colitis; inflammatory bowel disease; gut mucosal defense; lipopolysaccharides

INTRODUCTION

Inflammatory bowel diseases (IBD) encompass several chronic inflammatory diseases of the intestine, including ulcerative colitis (UC) and Crohn's disease (CD) (1, 2). These gut inflammatory conditions are associated with the clinical signs and symptoms of diarrhea, malabsorption, and impaired gut barrier function. IBD patients are also at increased risk for the development of cancer (3). The etiology of IBD remains unclear, but genetic and environmental factors along with chronic dysregulation of the host immune response to the gut flora appear to play key roles in its pathogenesis (1, 2, 4). In the human gut, bacterial products like lipopolysaccharides (LPS), unmethylated cytosine-phosphate-guanosine deoxyribonucleic acids (CpG DNA) and flagellins are known to elicit host immune responses that are necessary to maintain normal mucosal defense against invasion from opportunistic bacteria. However, dysregulation of the host immune response to the gut flora and/or inadequate protective mechanisms against gut-derived bacterial products may lead to excessive intestinal inflammation (1, 2, 4).

Recently, we found that intestinal alkaline phosphatase (IAP) (EC 3.1.3.1), a small intestinal brush-border enzyme, functions as a gut mucosal defense factor. We showed that IAP provided resistance to bacterial invasion when the intestine was subjected to a local or distant ischemic injury (5). Loss of IAP expression is also associated with increased LPS toxicity in zebrafish (6). Most recently, decreased IAP expression in UC patients was shown to be correlated with gut inflammation (7), and Lukas *et al.* demonstrated that naso-duodenal delivery of cIAP to UC patients resulted in improvement in clinical and serological measures (8). Although the IAP enzyme is known to detoxify LPS (6, 9, 10), the role that IAP plays in intestinal inflammation has not been fully elucidated. Oral IAP treatment recently was shown to be protective in a DSS model of gut inflammation in rats (7). However, this study employed a single exposure to DSS that is generally considered an acute injury model rather than a reflection of the more chronic inflammation seen in human IBD. As such, we sought to demonstrate a potential therapeutic role for orally administered cIAP in the context of two independent mouse models for chronic colitis: mice chronically treated with DDS and mice deficient in Wiskott-Aldrich Syndrome protein (WASP), which develop spontaneous colitis, a process accelerated by irradiation. Chronic oral administration of 2% DSS disrupts the intestinal epithelial cell barrier and consistently induces focal mucosal injury in rodents that mimics UC-like disease found in humans (11, 12). The WASP, an intracellular signaling molecule expressed in hematopoietic cells, is intimately involved in actin cytoskeletal rearrangement. WASP knockout (KO) mice are known to harbor several immune cell defects including defects in T cell activation (13, 14), quantitative and qualitative defects in regulatory T cells (Tregs) (15–18), and leukocyte migratory defects (19, 20). Starting at four months of age, these mice develop chronic

spontaneous colitis that is associated with a Th2-skewed cytokine profile (21). Exposure of these mice to sub-lethal irradiation (700 rads) leads to an earlier onset of colitis with a more uniform disease induction (unpublished data). Because of their close resemblance to human chronic colitis, we chose these two mouse models to test the potential therapeutic application of IAP in IBD.

Here, we report that endogenous IAP likely protects the host from IBD, and oral supplementation of IAP ameliorates clinical signs and symptoms of IBD in two mouse models of chronic colitis supporting a potential therapeutic value of IAP to prevent/treat human IBD.

MATERIALS AND METHODS

Animals

Specific pathogen-free IAP-KO (*Mus musculus* C57BL/6) mice (22) were obtained from the Burnham Institute Medical Research (La Jolla, CA) and bred at the Massachusetts General Hospital (MGH, Boston, MA) animal facility to create homozygous IAP-KO, heterozygous, and wild-type C57BL/6 (WT) littermates. Genotype was confirmed by PCR analysis (22). WASP-KO (129 SvEv background) mice were generated as previously described (13) and maintained in a specific pathogen-free (SPF) environment at MGH in accordance with the guidelines of the Committee on Animals of Harvard Medical School (Boston, MA). All experiments were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and according to regulations of the Subcommittee on Research Animal Care of the MGH and the National Institutes of Health (publication no. 85-23, NIH, 1985).

Induction of Chronic Colitis by DSS in IAP-KO and WT Mice and Treatment with cIAP

Experimental chronic colitis was induced by cyclic administration of 2% DSS (molecular weight, 36–44 kD, MP Biomedicals, Solon, OH) in the drinking water. For each experiment, mice were divided into four equal groups (n = 5) receiving DSS and/or cIAP as described below: (1) No DSS, No cIAP; (2) No DSS, Plus cIAP; (3) Plus DSS, No cIAP; (4) Plus DSS, Plus cIAP. Both IAP-KO and WT mice received four cycles of DSS given *ad libitum* for 7 days followed by a 7-day DSS-free interval during which mice were treated with either cIAP (New England Biolabs, Ipswich, MA) or ‘vehicle for cIAP’ (50 mM KCl, 10 mM Tris-HCl (pH 8.2), 1 mM MgCl₂, 0.1 mM ZnCl₂, and 50% glycerol). Stool samples were analyzed to ensure the intraluminal activity of cIAP. The control group received drinking water without DSS but with the cIAP vehicle throughout the experimental period. Body weight of each animal was regularly monitored.

Induction of Spontaneous Chronic Colitis in WASP KO Mice by Sub-Lethal Irradiation

During bone marrow transplantation experiments, it was noted that lethal irradiation of WASP-KO mice led to early mortality, likely from induction of severe colitis (23). Sub-lethal irradiation, however, did not lead to mortality but did accelerate colitis onset with a more uniform disease induction (unpublished data). In these experiments, 8–10 week-old WASP-KO mice were sub-lethally irradiated at 700 rads. Mice then received either vehicle or cIAP in the drinking water continuously for 6 weeks, after which time the mice were sacrificed for histological and biochemical analyses.

Histological Injury Score

For histopathological analysis in DSS-induced chronic colitis, colons were divided into distal, middle and proximal portions and were fixed in 10% formalin, embedded in paraffin, sectioned (4 µm), stained with haematoxylin and eosin (H&E), and examined by an independent investigator (pathologist) in a blinded fashion. A previously published grading

system was used to evaluate the severity of inflammation, depth of injury/inflammation, and crypt damage (24). A score of 0–4 was assigned to each of these parameters. Total score of each parameter was further multiplied by a factor reflecting the percentage of tissue involved (x1, 0 to 25%; x2, 26 to 50%; x3, 51 to 75%; x4, 76 to 100%). The sum of the scores from these three parameters was defined as the histological injury scores obtained in three sections (25). For WASP KO colitis, mid and distal portions of the colon were harvested for H&E staining in a similar fashion. Scoring was again performed by the same independent investigator (pathologist) in a blinded fashion using a scale previously published (21). Briefly, scores ranged from 0–8 (0–3 for wall thickening, 0–3 for extent of lamina propria infiltration, and 0–2 for presence of crypt abscesses).

Colon Organ Culture and Analysis of Cytokine Production in IAP-KO and WT Mice

One cm of the middle colonic segment was washed with serum free medium (RPMI-1640, Invitrogen, Carlsbad, CA) containing penicillin and streptomycin (Invitrogen) and then minced and placed in 1 ml of RPMI-1640 medium containing 10% fetal bovine serum (FBS, Lonza, Allendale, PA), penicillin and streptomycin in 24-well flat-bottom tissue culture plates and incubated for 24 h at 37°C in 5% CO₂, as previously described (26). Cell-free supernatant was collected after 24 h, and cytokine secretion (TNF- α and IL-6) was analyzed by enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, San Diego, CA) according to the manufacturer's protocol.

Analysis of Cytokine Production in WASP-KO Mice

Colonic lamina propria cells were isolated and cultured as described in Nguyen *et al.* (21). Supernatant was collected after three days of culture, and cytokine levels were analyzed by ELISA as described above.

Determination of Myeloperoxidase (MPO) Activity in Colon

MPO (EC 1.11.1.7) is a peroxidase enzyme mainly present in polymorphonuclear leukocytes and correlated with the quantity of neutrophil infiltration into tissues (27). Colonic tissues were rinsed with cold phosphate buffered saline (PBS, Invitrogen), blotted dry, immediately frozen on dry ice, and stored at –80°C. The MPO assay was performed using the *o*-dianisidine dihydrochloride method (27). Colonic samples were thawed, weighed, and homogenized (Sonic Dismembrator Model 300, Fisher Scientific, Pittsburgh, PA) in 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% ultra hexadecyltrimethylammonium bromide (Sigma, St. Louis, MO). After homogenization, samples were centrifuged at 13,000 rpm for 15 minutes at 4°C. The reactions were initiated by mixing and incubating the supernatant (100 μ L) at 20°C for 10 minutes with a solution containing 50 mM potassium phosphate, 30 mM of *o*-dianisidine dihydrochloride, and 20 mM hydrogen peroxide in a final volume of 300 μ L. Reactions were terminated by the addition of 2% sodium azide (50 μ L). Absorbance was measured at 460 nm in a spectrophotometer and enzyme activity was expressed as the amount of enzyme necessary to produce a change in absorbance of 1.0 unit per minute per milligram of wet tissue.

Statistical Analysis

Statistical calculations were performed using the InStat software package (Graphpad software, La Jolla, CA). Duplicate or triplicate samples were used for all assays. All experiments were repeated two or three times, and data were analyzed using ANOVA, unpaired Student *t*-test, and the Tukey-Kramer multiple-comparisons posttest. *P*-values (*, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001) are considered statistically significant.

RESULTS

Oral cIAP Treatment Reduces Weight Loss in IAP-KO and WT Mice with DSS-Induced Chronic Colitis

DSS-treated IAP-KO and WT mice showed significant weight loss at the end of the DSS cycles (week 8) of $-12.91\% \pm 2.7\%$ and $-21.3\% \pm 1.05\%$, respectively, compared to their initial body weight (Fig. 1, A and B). Interestingly, less weight loss was seen in the DSS-treated IAP-KO mice when compared to DSS-treated WT mice, perhaps reflecting the tendency toward obesity previously described for these KO mice (22). In both the WT and KO animals exposed to DSS, cIAP treatment was associated with less weight loss (DSS/KO-cIAP *vs.* DSS/KO: $-12.9 \pm 2.7\%$ *vs.* $-5.83 \pm 2.82\%$ and DSS/WT-cIAP *vs.* DSS/WT: $-21.3\% \pm 1.05\%$ *vs.* $-5.19 \pm 2.01\%$, $P \leq 0.05$). Control groups of untreated WT and IAP-KO mice showed no clinical signs of intestinal inflammation and had normal weight gain over time. We observed less weight gain in cIAP-treated WT animals compared to the control animals (no cIAP treatment), however, the difference was not statistically significant.

Histological Assessment Reveals Reduction of Inflammation in DSS-Induced Chronic Colitis in cIAP-Treated IAP-KO and WT Mice

Semiquantitative microscopic colitis scores (Fig. 2A) showed that in DSS-administered IAP-KO mice, the severity of colitis (as assessed by total score, inflammation, depth of injury, and presence of crypt abscesses) was significantly higher than that in DSS-treated WT mice (DSS/KO *vs.* DSS/WT: 52 ± 3.87 *vs.* 28.8 ± 6.66 , $P < 0.0001$). Furthermore, oral cIAP treatment (300 units/day) attenuated the disease in both groups (DSS/KO-cIAP *vs.* DSS/WT-cIAP: 30.7 ± 6.01 *vs.* 18.7 ± 5.03 , $P < 0.05$). Mice in control groups without DSS demonstrated no colitis (data not shown). The overall DSS-induced injury score was higher in the distal segment followed by the middle and proximal segments of the colon in all mice treated with DSS, and cIAP treatment reduced the injury score in all segments (Fig. 2B).

Oral cIAP Treatment Rescues IAP-KO Mice from DSS-Induced Chronic Colitis

Histological analyses of sections of control, untreated WT and IAP-KO mice did not show any signs of inflammation (Fig. 3, A and B). Cyclic oral administration of 2 % DSS in the drinking water resulted in chronic colitis with a marked increase in the thickness of the muscularis propria, infiltration of inflammatory cells into the lamina propria, shortening of colonic crypts, and, in some sections, loss of crypts and goblet cells in both WT and IAP-KO mice colons (Fig. 3, C and D). Interestingly, mucosal injury was more apparent in chronic DSS-treated IAP-KO mice (Fig. 3D) compared to their WT counterparts (Fig. 3C). Specifically, DSS-administered IAP-KO mice showed loss and shortening of colonic crypts, severe inflammation in the lamina propria, crypt abscesses, and depletion of goblet cells to a greater degree leading to higher total histological injury score (also see Fig. 2). However, oral administration of cIAP led to a marked reduction in infiltration of inflammatory cells, submucosal edema (arrows), and mucosal damage (Fig. 3, E and F) resulting in a significant reduction ($P < 0.001$) of histopathological injury scores as shown in Fig. 2.

The Production of Pro-Inflammatory Cytokines Is Reduced in Colonic Tissue of IBD Mice Treated with cIAP

To determine the effect of cIAP on pro-inflammatory cytokine expression, colonic IL-6 and TNF- α levels were determined by organ culture (see Materials and Methods). Levels of IL-6 in chronic DSS-administered IAP-KO mice were higher than in DSS-treated WT mice (DSS/KO *vs.* DSS/WT: 325.2 ± 25.7 *vs.* 251.7 ± 27.7 pg/ml, $P < 0.05$), and treatment with oral cIAP significantly reduced IL-6 levels in both groups (DSS/KO-cIAP *vs.* DSS/WT

+cIAP: 201.1 ± 32.4 vs. 158.9 ± 22.6 pg/ml, $P < 0.05$) (Fig. 4A). TNF- α levels were also elevated (Fig. 4B) significantly in DSS-administered IAP-KO (DSS/KO vs. KO: 992.9 ± 63.2 vs. 158.5 ± 26.5 pg/ml, $P < 0.001$) and WT mice (DSS/WT vs. WT: 907.2 ± 144.4 vs. 129.4 ± 15.6 pg/ml, $P < 0.001$), compared to non-DSS-treated controls. However, no significant difference in TNF- α levels was seen between the two groups, but treatment with oral cIAP significantly reduced the TNF- α levels in both IAP-KO and WT mice (DSS/KO +cIAP vs. DSS/WT+cIAP: 677.2 ± 141.1 vs. 568.4 ± 74.9 pg/ml, $P < 0.05$).

Myeloperoxidase (MPO) Activity Is Reduced in the Colon of DSS-Induced Chronic Colitis in cIAP-Treated IAP-KO and WT Mice

DSS-induced colonic inflammation can be quantitatively determined by assessment of MPO activity (27). Of note, previous studies have shown that MPO activity is higher in DSS-induced acute colitis compared with DSS-induced chronic colitis (28, 29). As shown in Fig 5, compared to the controls, cyclic administration of 2% DSS was associated with a significant ($P < 0.05$) increase in MPO activity in both IAP-KO and WT groups. However, the increase in MPO activity was significantly higher in the KO mice compared to the WT mice (14.29 ± 0.83 vs. 9.23 ± 1.60 U/mg tissue, $P < 0.05$). DSS-induced increases in MPO activity were reduced significantly by oral administration of cIAP in both groups (KO vs. WT: 10.26 ± 0.54 vs. 6.70 ± 0.51 U/mg tissue, $P < 0.05$). These findings suggest that cIAP may exert anti-inflammatory effects in this chronic DSS model at least partly by reducing neutrophil infiltration.

Oral cIAP Treatment Attenuates Colitis in Irradiated WASP-KO Mice

Six weeks after irradiation, WASP-KO mice manifested spontaneous colitis as shown in Fig. 6A. However, 6 weeks of treatment with cIAP attenuated this irradiation-induced colitis (WASP-KO-cIAP vs. WASP-KO+cIAP: 6.25 ± 0.34 vs. 3.30 ± 0.53 , $P < 0.001$) (Fig. 6A). Shown in Fig. 6B are representative sections of two independent experiments of irradiated WASP-KO mice treated with vehicle or cIAP for 6 weeks. Histopathological analysis revealed that irradiated WASP-KO developed similar features, such as crypt elongation, epithelial cell hyperplasia, loss of goblet cells, and inflammatory cell infiltration into the lamina propria (Fig. 6B, left panel) as unmanipulated WASP-KO mice described previously (21). In contrast, cIAP-treated animals had minimal thickening of colonic crypts with less inflammatory cell infiltration into the lamina propria, and the overall mucosal architecture was maintained (Fig 6B, right panel).

Treatment with cIAP Reduces Cytokine Production in WASP-KO Mice with Chronic Colitis

Cultured colonic lamina propria (LP) leukocytes isolated from WASP-KO mice have been shown to secrete excessive amounts of IL-4 and IL-13 in addition to IFN- γ (21). Therefore, in the current study, colonic lamina propria cells were isolated and analyzed for their cytokine production. Stimulated LP cells from irradiated WASP-KO mice secreted elevated amounts of IL-4 and IFN- γ (Fig. 7, A and B). In contrast, stimulated LP cells from cIAP-treated irradiated WASP-KO mice had significantly reduced levels of these cytokines (IL-4: WASP-KO-cIAP vs. WASP-KO+cIAP: 2430.5 ± 995.0 vs. 449.4 ± 75.7 , $P < 0.001$; and IFN- γ : WASP-KO-cIAP vs. WASP-KO+cIAP: 47306.9 ± 11782.0 vs. 6105.8 ± 794.5 , $P < 0.05$).

DISCUSSION

In the present study, we showed that cIAP exerts protective effects in two independent mouse models of chronic colitis. In our first model, we found that IAP-KO mice displayed increased susceptibility to chronic DSS colitis compared to their WT littermates. These results indicate that endogenous IAP plays a role in protecting the host from excessive

intestinal inflammation. In addition, exogenous oral administration of cIAP significantly attenuated disease in both IAP-KO and WT groups, pointing to the potential therapeutic role for this enzyme. In our second model, cIAP again showed therapeutic benefit in preventing the spontaneous colitis that occurs in WASP-KO mice, which is accelerated by irradiation. MPO activity and pro-inflammatory cytokine levels correlated with the degree of histological inflammation in our models of colitis, supporting a beneficial role for IAP. Interestingly, while endogenous IAP limits fat absorption (22), IAP-KO mice receiving oral cIAP did not show any significant reduction in weight compared to the control group receiving no cIAP (Fig. 1A). However, we observed a trend of weight loss in WT mice receiving oral cIAP compared to the control WT animals receiving no cIAP (Fig. 1B). We postulate that higher doses of cIAP might be able to significantly reduce weight gain. Further studies will be required to determine the effects of oral IAP administration on weight gain.

The suggestion from our data that endogenous IAP has a protective role against gut inflammation is consistent with the findings in Tuin *et al.* (7) that endogenous IAP levels were decreased in inflamed tissue compared to non-inflamed tissue in IBD patients. Moreover, endogenous IAP levels were also significantly reduced in non-inflamed tissue from IBD patients compared with tissue from normal healthy controls (7), indicating that this reduction is potentially an early marker for IBD and may represent a causal mechanism of the disease. It is possible that some patients are more susceptible to IBD because they genetically have less IAP expression or activity, which might alter responsiveness to bacterial products like LPS, ultimately leading to intestinal inflammation. However, in human populations, there is little known about IAP polymorphism or variability in endogenous levels of this enzyme. Clearly, further human studies are needed to address this interesting question.

Rescue of increased severity in DSS-treated IAP-KO mice with exogenous cIAP confirms that the exacerbated colonic mucosal inflammation seen in IAP-KO mice compared to WT was secondary to the lack of IAP expression. In fact, exogenous oral administration of cIAP in IAP-KO mice resulted in re-epithelialization with normalized colonic mucosal architecture and less inflammatory cell infiltration (Fig. 3), higher body weights (Fig. 1) and marked reductions in the histological injury scores (Fig. 2). These findings are consistent with the finding in Tuin *et al.* (7) in the rat model of acute DDS colitis (4, 29). Our study investigated two independent models of chronic colitis, which more closely resemble human IBD. In the chronic DSS model, colitis involves an immune response characterized by pro-inflammatory cytokines and immunoglobulin G immune complex formation response (29–31). In the WASP-KO colitis model, colitis involves a quantitative and qualitative defect in regulatory T cells as well as defects in T cell activation and a Th2-skewed cytokine profile (21). A subset of human patients with defects in WASP also suffer from colitis (32, 33). Since these two models closely resemble the disease condition seen in ulcerative colitis, we believe our findings are clinically relevant and provide a foundation for the use of IAP in human UC patients. Indeed, the therapeutic effect of orally administered IAP in these mouse models is in line with the recent human trial in which UC patients benefited from naso-duodenal cIAP (8).

There may be therapeutic advantages of cIAP compared to other IBD therapies, since it is a naturally occurring enzyme with no known toxicity or serious side effects (8). It should be noted, however, that the single human trial was not a placebo-controlled, double-blinded study, and therefore, further clinical studies are needed to confirm the efficacy of IAP as a UC treatment. It is not known whether IAP could be a therapy for Crohn's disease.

The precise molecular mechanisms of how cIAP functions in these chronic colitis models are still unknown. However, we hypothesize that LPS detoxification may be one mechanism for cIAP's effect against colitis. LPS signaling, through TLR4 activation, can lead to pro-inflammatory cytokine expression and neutrophil recruitment. Given the ability of the IAP enzyme to detoxify LPS, it is possible that this detoxification results in a decrease in LPS/TLR4 signaling and therefore less inflammation.

In addition, IAP may also work through other targets, such as bacterial CpG DNA (34) and flagellin (34, 35). Our data have shown that cIAP can dephosphorylate unmethylated CpG DNA and block its pro-inflammatory cytokine effects *in vitro*, similar to its effects on LPS DNA (34). Interestingly, oral administration of bacterial CpG DNA has been shown to aggravate DSS-induced chronic colitis (36). Future studies will be required to determine whether dephosphorylation of CpG and/or flagellin by IAP leads to an attenuation of the intestinal inflammation in the DSS-induced chronic colitis model.

It should be noted that the normal expression of IAP is the highest in the proximal small intestine, whereas it is very low to nonexistent in the large intestine, the location of high bacterial titers and inflammation. However, we have previously shown that IAP enzyme travels distally through the gut and remains active even in the stool. As such, it is possible that when an imbalance occurs in the gut flora, the small intestine increases production of IAP (6) and the surfactant-like particles (37, 38) that are secreted into the gut lumen then travel to the large intestine where they may be most functional. Here IAP may encounter bacterial products like LPS, DNA, and flagellin and exert its effect of maintaining normal intestinal homeostasis (6).

Another possible mechanism of cIAP action may involve changing the gut microflora. We have found that IAP-KO mice have an altered flora and furthermore, that orally administered IAP can help restore the flora that is disrupted by antibiotics (unpublished observations). It is possible that differences in the flora contribute to the worsening DSS-induced chronic colitis in IAP-KO mice. DSS-induced colitis in WT mice has been shown to be ameliorated by treatment with antibiotics that are clinically effective in patients with IBD (30). In addition, DSS fails to induce colitis in germ-free mice (39). Therefore, IAP may be effective as a therapeutic agent against IBD through maintenance of normal gut flora homeostasis, though further studies are needed to confirm this theory.

Another potential mechanism involves control of pro-inflammatory cytokine secretion. A wide variety of animal models and human studies have shown that TNF- α and IL-6 are important pro-inflammatory mediators of IBD (30, 40, 41), and neutralization of these pro-inflammatory cytokines attenuates disease development in both humans and animals (42–44). Our analysis of pro-inflammatory cytokine production by colon culture revealed significant elevations of TNF- α and IL-6 in chronic DSS-treated IAP-KO mice (Fig. 4) compared with WT mice, and subsequent oral treatment with cIAP significantly reduced cytokine levels in both groups. In irradiated WASP-KO mice, elevated levels of IFN- γ and IL-4 were seen in stimulated LP cells (Fig. 7); similar to observations in unmanipulated WASP-KO mice (21), and oral treatment with cIAP significantly reduced these cytokine levels. Taken together, these findings suggest that cIAP, at least in part, may exert its anti-inflammatory effects through reduction of pro-inflammatory cytokines. In addition, we also assayed MPO activity as a measure of neutrophil infiltration (27). MPO activity has been widely used as a marker for gut inflammation (24). Increases in MPO activity lead to formation of reactive oxygen species (ROS), which are thought to be important mediators in the pathogenesis of IBD-related mucosal damage (7). Analysis of tissues from chronic DSS-treated IAP-KO mice revealed higher levels of MPO enzyme activity compared to WT mice. Oral treatment with cIAP significantly suppressed MPO activity in both IAP-KO and WT

mice (Fig. 5), indicating less neutrophil infiltration in the colon tissue and hence less inflammation. These results highlight the ability of cIAP to inhibit neutrophil migration and IBD progression in a chronic DSS-induced colitis model.

To summarize, the present work provides evidence that cIAP exerts protective effects against two independent mouse models of chronic colitis, either through detoxification of microbial products like LPS, unmethylated CpG DNA, and flagellins and/or by suppression of pro-inflammatory cytokine release. Though detailed mechanism of the effect of cIAP in IBD remains to be more fully elucidated, it appears that oral administration of cIAP may represent a novel effective therapy for of human IBD.

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Abbreviations

cIAP	calf intestinal alkaline phosphatase
CpG DNA	cytosine-phosphate-guanosine deoxyribonucleic acid
DSS	dextran sulfate sodium
IAP	intestinal alkaline phosphatase
IAP-KO	IAP knockout
IBD	inflammatory bowel disease
LPS	lipopolysaccharides
TNF-α	tumor necrosis factor alpha
WASP	Wiskott–Aldrich syndrome protein

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FIGURE 1.

Effects of cIAP on the body weight of IAP-KO and WT mice with DSS-induced chronic colitis. Change in body weight expressed as percentage of initial weight during cyclic administration of 2% DSS in drinking water. Significantly less weight loss was seen in both IAP-KO (A) and WT (B) groups receiving DSS+cIAP compared to DSS alone groups (*, $P \leq 0.05$). No significant differences were detected between cIAP and the control groups. Statistical analyses were carried out using Tukey-Kramer multiple-comparisons posttest. Data are expressed as the mean $\pm$ standard deviation (SD). Legend: Control = no DSS, no cIAP; +cIAP, = no DSS, plus cIAP; +DSS = plus DSS, no cIAP; +DSS+cIAP = plus DSS, plus cIAP.

FIGURE 2.

(A) Histological injury score of DSS-induced chronic colitis in IAP-KO and WT mice. Highest colitis score demonstrated in DSS-treated IAP-KO mice with attenuation of disease by exogenous IAP (***, $P \leq 0.001$) but not to the level of WT mice also treated with IAP (**, $P \leq 0.01$). (B) Histological injury score from distal, middle and proximal parts of colon of IAP-KO and WT mice. Severity of histological abnormalities is the highest distally. Statistical analyses were carried out using unpaired Student *t*-test. Data are expressed as the mean $\pm$ standard error (SEM).

FIGURE 3.

Histological evaluation of DSS-induced chronic colitis in IAP-KO and WT mice.

(Structures: **e**, epithelial disruption; **i**, inflammatory infiltrate; **m**, lamina muscularis mucosa; **s**, submucosal edema; **t**, thickening in lamina muscularis propria; **Re**, re-epithelialization: magnification 200X). Results are representative of two independent experiments.

Representative H&E staining reveal normal histology in WT (A) and IAP-KO (B) control mice without DSS. (C) DSS-treated WT colon. (D) More extensive mucosal ulceration, leading to complete loss of colonic crypts with inflammatory mononuclear cell infiltration in IAP-KO mice compared with WT (C) mice colons. Treatment effect of cIAP in DSS-induced chronic colitis in WT (E) and IAP-KO (F) mouse colons. cIAP-treated colons had preserved colonic architecture with less inflammatory cell infiltration and no submucosal edema (arrows) compared with non-treated colons (magnification 100X).

FIGURE 4.

Cytokine production in colons of DSS-induced chronic colitis in IAP-KO and WT mice. Elevated levels of IL-6 (A) and TNF- α (B) were found in colons of DSS-induced chronic colitis in IAP-KO mice compared with WT mice. Oral treatment with cIAP (300 units/day) downregulates pro-inflammatory cytokine release in both groups. *, ($P < 0.05$) significantly different from vehicle-treated group. Statistical analyses were carried out using unpaired Student *t*-test. Data are expressed as the mean $\pm$ standard error (SEM).

FIGURE 5.

Myeloperoxidase (MPO) activity in colons of DSS-induced chronic colitis in IAP-KO and WT mice. Elevated MPO activity were found in colons of DSS-induced chronic colitis in IAP-KO mice when compared with WT mice. However, DSS-induced increases in MPO activity were reduced significantly by oral administration of cIAP (300 units/day) in both groups ($P < 0.05$). Statistical analyses were carried out using unpaired Student *t*-test. Data are expressed as the mean $\pm$ standard error (SEM).

FIGURE 6.

Histological injury score and evaluation of irradiated WASP-KO mice treated with vehicle or cIAP for six weeks. (A) Six-week treatment with cIAP attenuated histological colitis score in irradiated WASP-KO mice compared with vehicle-treated group ($n=2$, ***, $P < 0.001$). (B) Displayed are representative sections of two independent experiments of irradiated WASP-KO mice treated with vehicle or cIAP for 6 weeks (objective: X100 and X200).

FIGURE 7.

Pro-inflammatory cytokine levels secreted by cultured colonic LP cells. Cells from irradiated WASP-KO mice receiving vehicle secreted markedly elevated amounts of IL-4 as well as INF- γ , whereas 6 weeks of treatment with oral cIAP significantly reduced these cytokine levels. Results shown are mean $\pm$ SEM pooling from 2 different experiments. *, ($p < 0.05$) significant difference between irradiated WASP-KO mice treated with vehicle or with cIAP. LP cells were either unstimulated (US) or stimulated with phorbol myristate acetate (PMA) + ionomycin (P+I). Statistical analyses were carried out using unpaired Student *t*-test. Data are expressed as the mean $\pm$ standard error (SEM).