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RESEARCH ARTICLES

L-Tryptophan exhibits therapeutic function in a porcine model of dextran sodium sulfate (DSS)-induced colitis☆

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Abstract

Conventional therapies for the treatment of inflammatory bowel disease (IBD) have demonstrated limited efficacy and potential toxicity; therefore, there is a need for novel therapies that can safely and effectively treat IBD. Recent evidence has indicated that amino acids may play a role in maintaining gut health. L-Tryptophan has been shown to reduce oxidative stress and improve neurological states. The objective of this study was to assess the therapeutic effects of L-tryptophan in a porcine model of dextran sodium sulfate (DSS)-induced colitis. DSS was administered to piglets via intragastric catheter for 5 days followed by tryptophan administration at 80% of the daily recommended intake. The severity of colitis was assessed macroscopically and histopathologically, and intestinal permeability was monitored in vivo by D-mannitol analysis. The effect of tryptophan on the local expression of key mediators of inflammation and IBD pathogenesis was examined at the protein and gene expression levels. Supplementation with tryptophan ameliorated clinical symptoms and improved weight gain to feed intake conversion ratios. Histological scores and measurements were also improved, and gut permeability was notably reduced in tryptophan-supplemented animals. Moreover, tryptophan reduced the expression of the pro-inflammatory cytokines tumor necrosis factor-α, interleukin (IL)-6, interferon (IFN)-γ, IL-12p40, IL-1β and IL-17, as well as IL-8 and intracellular adhesion molecule-1, and resulted in increased expression of apoptosis initiators caspase-8 and Bax. These results demonstrate that L-tryptophan supplementation can reduce inflammation and enhance the rate of recovery in DSS-induced colitis and may be an effective immunomodulating agent for the treatment of IBD.

Keywords: Inflammatory bowel disease (IBD); Colitis; Dextran sodium sulfate (DSS); L-Tryptophan; Therapeutic; Inflammatory cytokine expression

1. Introduction

Inflammatory bowel diseases (IBDs), such as Crohn's disease and ulcerative colitis, are chronic relapsing inflammatory disorders of the gastrointestinal tract [1]. The precise pathogenesis of IBD is unknown; however, recent literature suggests that there is an exaggerated immune response to enteric bacteria with the influence of environmental and genetic factors [2,3]. In healthy individuals, the mucosal immune system senses the local microenvironment and recognizes and avoids reacting to commensal flora while providing immunity against harmful pathogens [4]. However, IBD-susceptible individuals lack the tolerance of the mucosal immune system towards enteric bacteria, resulting in an auto-inflammatory response [2]. This dysregulation of mucosal immunity in the gut of IBD patients causes an overproduction of inflammatory cytokines and trafficking of effector leukocytes into the intestine, thus leading to uncontrolled intestinal inflammation and damage to the epithelial barrier [5,6]. Clinical manifestations of IBD include abdominal pain, diarrhea often with blood, weight loss [7], increased gut permeability [8] and shortening or loss of the crypt [9]. Conventional IBD therapy typically involves pharmacological agents such as aminosalicylates [10], corticosteroids [11] and immunosuppressive drugs such as azathioprine [10,11]; however, these treatments have demonstrated variable efficacy, adverse side effects and potential long-term toxicity [10,12,13]. Therefore, the need for alternative therapeutic strategies is of utmost importance.

Amino acids are key regulators of metabolic pathways, and recent evidence has indicated additional roles for amino acids in maintaining gut health [14]. Tryptophan is the least prevalent essential amino acid in mammals. It serves as a building block for proteins and possesses various nonnutritional functions potentially important for IBD treatment [15]. Recent literature shows the immunosuppressive function of tryptophan via tryptophan depletion and through tryptophan catabolites. Pro-inflammatory cytokines, such as interferon (IFN)-γ, activate the rate-limiting tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (IDO) [16], which depletes local tryptophan supply to surrounding cells. Tryptophan

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depletion limits free tryptophan necessary for proliferating T cells and activates the general control non-derepressible-2 kinase to trigger cell-cycle arrest [17] and apoptosis [18,19] via the integrated stress response [20,21]. Furthermore, tryptophan catabolites, such as L-kynurenine, quinolic acid, kynurenic acid and 3-hydroxyanthranilic acid, are able to inhibit proliferation of CD4+ and CD8+ T cells, particularly activated T-helper (Th) 1 cells [18]. Tryptophan's antioxidant properties may also aid in ameliorating oxidative-stress-related pathophysiology in IBD patients [22,23]. The indole ring side group of tryptophan acts as a very efficient antioxidant in the biological system and provides protection against lipid and protein peroxidation [24,25].

In the present study, we examined the ability of L-tryptophan to reduce dextran sodium sulfate (DSS)-induced colitis symptoms and pathology and evaluated the effect of tryptophan supplementation on local gene expression using a porcine model of experimental colitis.

2. Materials and methods

2.1. Animals and experimental design

Four- to five-day-old Yorkshire piglets were obtained from the University of Guelph Arkell Swine Research Station (Guelph, ON) and were housed individually in metal floor pens with rubberized floors in a controlled 12-h light/dark cycle room. Room temperature was maintained at 26°C and supplemented with heating lamps. Piglets were fed a commercial milk replacer formula diet (Soweena Litter Life, Merrick's Inc., Wisconsin) three times a day, similar to their ad libitum intake level. Animals were surgically fitted with an intragastric catheter (Micro-Renathane, O.D., 0.8 mm; Braintree Scientific, Inc., Braintree, MA). The catheter was anchored to a silicone patch (about 8×12 mm; Access Technologies, Skokie, IL), which was further sutured onto the gastric wall with approximately 30 mm inserted inside the gastric lumen. Each animal was fitted in a custom-made vest with a dorsal pocket for temporary storage of the exterior segment of the catheter. Piglets were randomly assigned into one of three groups and administered DSS or saline, followed by tryptophan or saline, via intragastric catheter. At the end point, animals were sedated through an inhaled anesthetic, isoflurane (Aerrane; Anaquest, Madison, WI), and euthanized via an intracardiac injection of Euthanol (pentobarbital) at 0.3 ml/kg·body weight (BW). Colon tissues were immediately rinsed in cold saline solution (154 mM, pH 7.4) containing 0.1 mM phenylmethyl sulfonyl fluoride (PMSF) (Sigma-Aldrich, St. Louis, MO) and sampled for histology or flash frozen in liquid nitrogen for cytokine analysis. All procedures were carried out in accordance with the Canadian Council of Animal Care Guide to the Care and Use of Experimental Animals.

2.2. Induction of colitis and treatment with L-tryptophan

To induce colitis, piglets in the positive control (Pos) group and tryptophan treatment (Trp) group received DSS at 1.25 g·DSS/kg·BW·day (MW, 36,000–50,000; MP Biomedicals, Solon, OH) in sterile saline for 5 days. The negative control (Neg) group received an equal volume of sterile saline. The DSS dose was determined from previous reports of DSS-induced porcine colitis models and was calculated based on the animals' weight on the first day of the DSS infusion period [26,27]. Following 5 days of DSS or saline administration, animals were given either L-tryptophan (Degussa-Hüls AG, Frankfurt am Main, Germany) (Trp group), solubilized in sterile saline at 80% of the daily recommended feed intake (110 mg/kg.BW.day [28], or saline (Neg and Pos groups) for 5 days. Animals in the Neg and Pos groups were infused with L-alanine for isonitrogen and isoenergetic balance. The infusion daily dose of true digestible amino acids was 0.115 g/kg·BW·day for the Trp group and 0.110 g/kg·BW·day for the Neg and Pos groups supplemented with alanine.

2.3. Growth performance and symptomatic parameters

Daily clinical evaluations included measurement of BW, feed intake, observation of stool consistency and presence of blood and observation of overall animal well-being.

2.4. In vivo intestinal permeability analysis

D-Mannitol was used to assess in vivo intestinal permeability as previously described [29]. Briefly, piglets were infused with 0.6 g/kg·BW of D-mannitol (Sigma-Aldrich), in a total volume of approximately 13 ml/kg·BW. Blood was collected into heparinized tubes (Sigma-Aldrich) at 0, 35 and 70 min, and plasma was collected by centrifugation at 800×*g* for 5 min. Background interference by polymer organic compounds was reduced by boiling tubes for 3–5 min followed by centrifugation at 21,000×*g* for 60 min. D-Mannitol concentrations were determined spectrophotometrically at 340 nm by measuring NADH yield produced by incubation with D-mannitol dehydrogenase (Megazyme International Ireland Ltd., Co., Wicklow, Ireland) for 150 min at 40°C. Concentrations were calculated from a D-mannitol standard curve.

2.5. Histopathological parameters

Colon tissues were fixed in 10% formalin for 24 h and stored in 70% ethanol until tissues were ready to be sectioned. Approximately five to six colon tissue cross sections from each animal were embedded in paraffin blocks and stained with hematoxylin and eosin (H&E). The total crypt depth, mature and immature crypt cell length and muscle thickness were measured using the Openlab software (Improvision, Coventry, UK) and Leica DMR microscope (Leica Microsystems GmbH, Wetzlar, Germany). The immature and mature measurements were subjectively defined as follows: the immature crypt portion was measured from the base of the crypt that stained dark with H&E due to DNA replication and the mature crypt portion included the apical portion of the crypt that stained light with H&E staining [30]. The total crypt length was the sum of immature and mature crypt measurements. Only the five longest consecutive villi were used for the calculation of the mean in order to avoid the bias of the missing third dimension.

Histological colon inflammation was blindly graded by an experienced animal pathologist (University of Guelph, Ontario Veterinary College, Guelph, ON). Four different features were graded for colitis using assessment criteria adopted from Dieleman et al. [9]. Briefly, for inflammation: 0=none, 1=slight, 2=moderate and 3=severe. For extent: 0=none, 1=mucosa, 2=mucosa and submucosa and 3=transmural. For regeneration: 0=complete regeneration or normal tissue, 1=almost complete regeneration, 2=regeneration with crypt depletion, 3=surface epithelium not intact and 4=no tissue repair. Lastly, for crypt damage: 0=none, 1=basal 1/3 damaged, 2=basal 2/3 damaged, 3=only surface epithelium intact and 4=entire crypt and epithelium lost. Furthermore, the percentage-involvement of each feature was scored: 1=1–25%, 2=26–50%, 3=51–75% and 4=76–100%. Each of the four features was multiplied by the percentage-involvement score to give a range of 0–12 for inflammation and extent and a range of 0–16 for regeneration and crypt damage.

2.6. Measurement of IL-6 and TNF-α by enzyme-linked immunosorbent assay (ELISA)

Concentrations of the cytokines interleukin (IL)-6 and tumor necrosis factor (TNF)-α in the colon were analyzed by ELISA. Colon tissues were ground in liquid nitrogen, and 2 g of pulverized tissue was homogenized in cold HBSS (HyClone, Logan, UT) containing 2 μg/ml *N*-tosyl-L-phenylalanine (Sigma-Aldrich), 2 μg/ml *N*-α-*p*-tosyl-L-lysine ketone (Sigma-Aldrich), 2 μl/ml leupeptin (Sigma-Aldrich), 2 μg/ml aprotinin (Sigma-Aldrich), 2 μg/ml pepstatin A (Sigma-Aldrich) and 0.2 mM PMSF (Sigma-

Table 1
Porcine primers used for real time RT-PCR

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)	Product (bp)	Accession no.
β-Actin	GGATGCAGAAGGAGATCACG	ATCTGCTGGAAGGTGGACAG	130	U07786
IL-6	AAGGTGATGCCACCTCAGAC	TCTGCCAGTACCTCCTTGCT	151	M86722
TNF-α	ATGGATGGGTGGATGAGAAA	TGGAAACTGTTGGGGAGAAG	151	X54001
IL-8	TGGCAGTTTTCCTGCTTTCT	CAGTGGGGTCCACTCTCAAT	154	M86923
IFN-γ	CCATTCAAAGGAGCATGGAT	GAGTTCACTGATGGCTTTGC	146	AY188090
IL-1β	CAAAGGCCGCCAAGATATAA	GAAATTCAGGCAGCAACAT	147	NM_214055
IL-17	TCATGATCCCACAAAGTCCA	AGTCCATGGTGAGGTGAAGC	146	NM_001005729
ICAM-1	GCCCAATTGAAGCTGAATGT	CACCTGGGTCTGGTTCTTGT	108	NM_213816
IL-12p40	TTTCAGACCCGACGAACTCT	CATTGGGGTACCAGTCCAAC	160	NM_214013
Caspase-8	AGATGACTCCGGAAGCTTGA	AGAGGCAGAGGCTCATGTGT	103	AY519263
cFLIP	GAGCAAGCCCCTAGGAATCT	GTCTTGGTGTTGGGGCATAC	172	NM_001001628
Bax	TAACATGGAGCTGCAGAGGA	AAAGTAGAAAAGCGCGACCA	133	AJ606301

Table 2
Comparison of growth performance of Pos, Neg and Trp treatment groups

	Neg (n=6)	Pos (n=8)	Trp (n=7)
Initial BW (kg)	2.35±0.09	2.52±0.09	2.27±0.17
Final BW (kg)	5.61±0.35	5.54±0.19	5.14±0.43
Daily BW gain (g/day)	169.03±15.27	140.92±8.49	159.08±13.25
Average formula intake (ml/day)	1136.17±88.75	1179.18±68.84	1068.50±91.65

Values represent mean±S.E.M.

Aldrich) using a PowerGen 700D Fisher Scientific Homogenizer (Thermo Fisher Scientific, Waltham, MA). The homogenized samples were centrifuged at 4°C for 15 min at 12,000×*g*. IL-6 and TNF-α concentrations were measured using Porcine IL-6 and TNF-α Quantikine ELISA kits according to the manufacturer's instructions (R&D Systems, Inc., Minneapolis, MN).

2.7. RNA isolation and regulation of gene expression by real-time reverse-transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from colon tissue using the Aurum Total RNA Mini Kit (Bio-Rad Laboratories, Inc., Hercules, CA) and first-strand cDNA synthesis was carried out using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Inc.), according to the manufacturer's instructions. Real-time PCR was carried out using iQ SYBR Green Supermix (Bio-Rad Laboratories, Inc.) on a MyiQ Single-Color Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.) using the following conditions: denaturation, 15 s at 95°C; annealing, 15 s at 56°C; and extension, 30 s at 72°C. Porcine primers (Table 1) were designed using Primer3 v. 0.4.0 [31] and synthesized by the University of Guelph Laboratory Services Molecular Biology Section (Guelph, ON).

2.8. Statistical analyses

Results are presented as mean values±S.E.M. Statistical analyses were performed using GraphPad Software (San Diego, CA). Comparisons between groups were performed with one-way analysis of variance followed by the Tukey–Kramer multiple comparison test. Intestinal permeability statistical analysis was accomplished by using SAS (SAS Institute, Inc., Cary, NC), and related linear regression analyses were conducted using the Fig.P program (Fig.P, 1993, Biosoft, Cambridge, UK). Differences in the slopes of the linear relationships between treatment groups were compared using the pooled t test. A P value less than .05 was considered to be statistically significant.

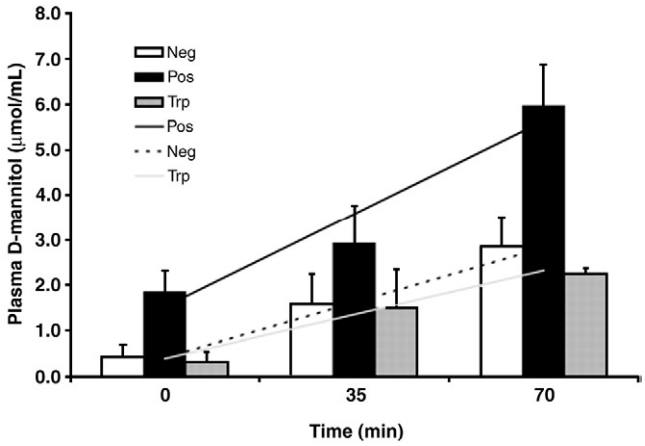

Fig. 2. Linear relationships between plasma D-mannitol concentration and the time post-intragastric infusion of D-mannitol in the Neg, Pos and Trp-treated piglets. Values represent mean±S.E.M for each of the marked time points, and duplicates of each sample were taken: n=6, Neg; n=8, Pos; n=7, Trp. For the Neg group, y=1.22 (±0.03) x, r^2=.99; for the Pos group, y=2.23 (±0.57)x, r^2=.94; and for the Trp group, y=0.85 (±0.38)x, r^2=.93; P<.05 for all parameter estimates. Differences in the slopes of linear relationships were observed between the Pos and Neg groups, P=.01; between the Pos and Trp groups, P=.003; and between the Neg and Trp groups, P=.021.

3. Results

3.1. Symptomatic parameters, growth performance and macroscopic observation

After 5 days of DSS infusion, severe and bloody diarrhea was observed. Following 5 days of tryptophan or saline administration, animals in the Trp group were free of severe and bloody diarrhea and had improved stool consistency when compared to the Pos group.

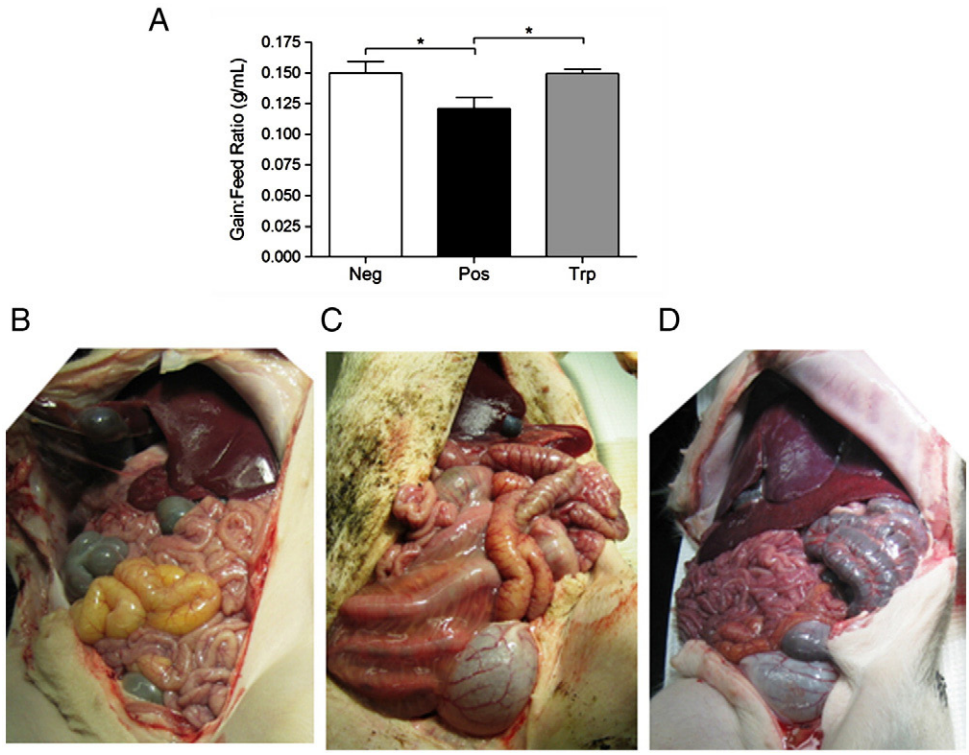

Fig. 1. Effect of tryptophan on DSS-induced colitis in piglets. (A) BW gain to feed intake ratio (in grams per milliliter) of Neg (n=6), Pos (n=8) and Trp (n=7) groups. Values indicate mean±S.E.M. Sample necropsy images of the intestine of (B) Neg, (C) Pos and (D) Trp piglets in the ventral position. *P<.05.

Animals in the Pos group had the lowest daily BW gain, despite a higher feed intake (Table 2), and significantly reduced BW gain to feed intake conversion ratio ($P<.05$) when compared to the Neg and Trp groups (Fig. 1A). The gross appearance of the intestine of the animals during necropsy is shown in Fig. 1B–D. Animals in the Pos group (Fig. 1C) showed macroscopic signs of acute inflammation including increased vascularization in the small and large intestine and thickening of the intestinal wall when compared to the Neg (Fig. 1B) and Trp (Fig. 1D) groups.

3.2. In vivo intestinal permeability analysis

Increased intestinal permeability is one of the etiological factors of IBD and, thus, can be used as a biomarker to assess the severity of small and large intestinal damage. The relationship between plasma D-mannitol concentration and time postinfusion was linear across all groups ($P<.05$; Fig. 2). The rate of D-mannitol uptake was used as an indicator of in vivo gut permeability and was determined from the slopes of the linear relationships by the increase in plasma D-mannitol concentration per minute. The rate of plasma D-mannitol increase (rate±S.E.M.) was significantly higher in the Pos group (2.23±0.57 μmol mannitol/ml plasma·min) than in both the Neg group (1.22±0.57 μmol mannitol/ml plasma·min; $P=.01$) and the Trp group (0.85±0.38 μmol mannitol/ml plasma·min; $P=.003$), suggesting that the administration of tryptophan aided in the restoration of gut barrier integrity.

3.3. Histopathological parameters of the colon

The severity of colonic inflammation was assessed by inflammation scoring and histological measurements (Figs. 3 and 4). When compared to the Neg (Fig. 3A) or the Trp (Fig. 3C) group, the colon of animals in the Pos group (Fig. 3B) had healed only partially after a 5-day recovery period. H&E-stained colon sections from Pos animals (Fig. 3B and D) showed distorted crypt architecture, infiltration of inflammatory cells into the mucosa and submucosa, crypt abscesses and cryptitis, whereas in the Trp group, there was a visible reduction in inflammatory cell

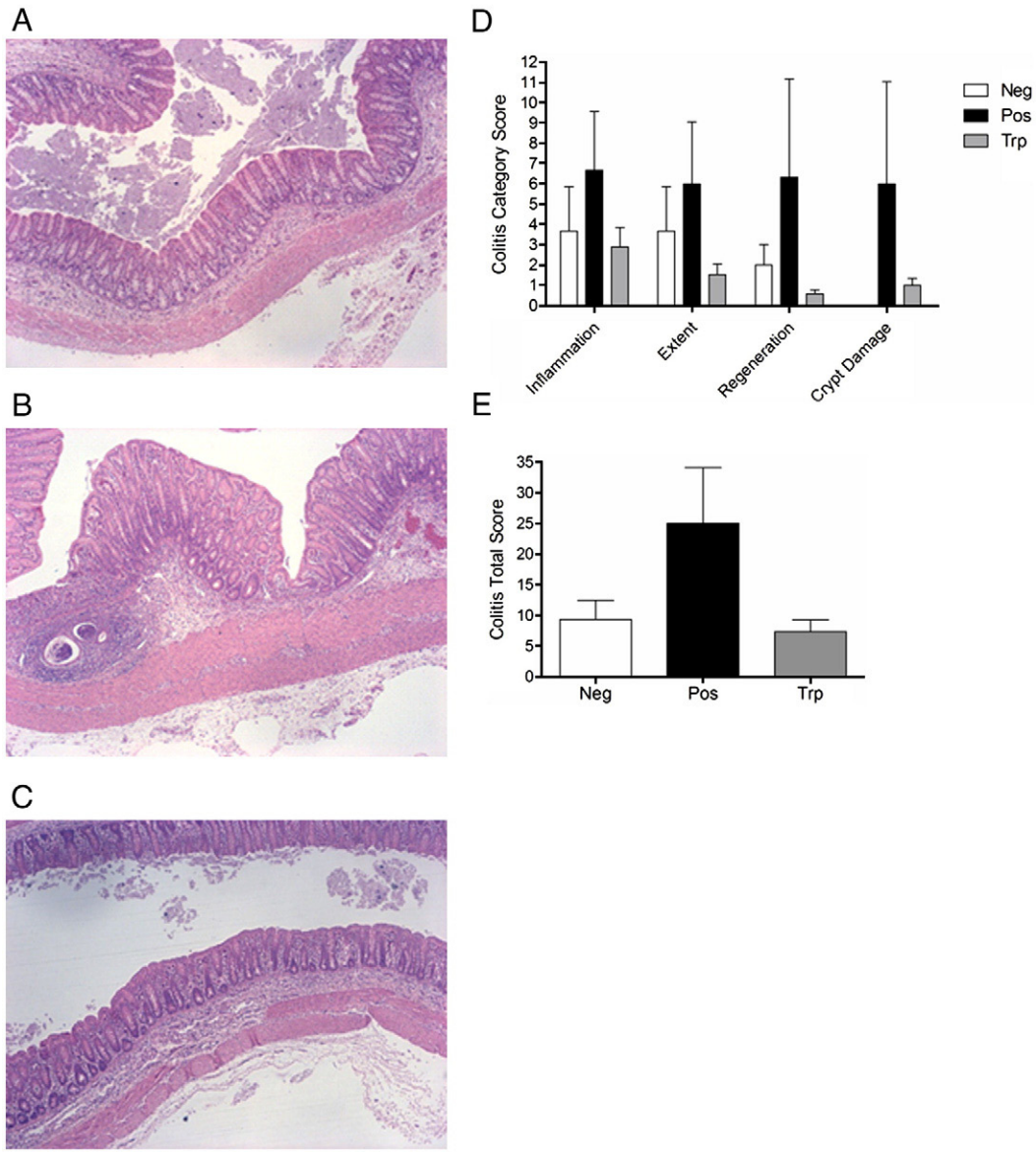

Fig. 3. Histopathological analyses of the colon after 5 days of intragastric DSS infusion followed by a 5-day period of recovery or tryptophan supplementation. Sample colon histological slides stained with H&E at ×50 magnification. Panel of slides indicate (A) Neg, (B) Pos and (C) Trp of sample histology images. Histological grading of colitis: (D) grading of inflammation, extent, regeneration and crypt damage according to Dieleman et al. [9]; (E) Sum of the category scores represented in Panel A. Values indicate mean±S.E.M., $n=3$ for all groups.

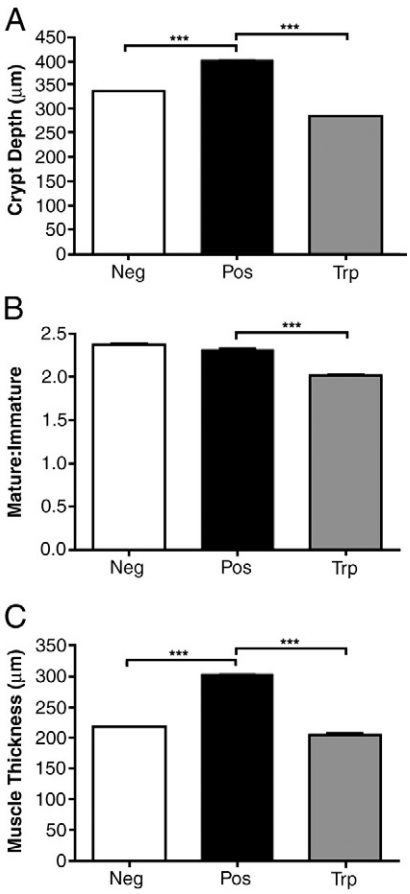

Fig. 4. Histological measurements of H&E-stained colonic cross sections of (A) crypt depth, (B) mature crypt cell-to-immature crypt cell ratio and (C) muscle thickness of Pos, Neg and Trp groups. Values indicate mean±S.E.M, $n=3$ for all groups. $^{***}P<.001$.

infiltration and crypt damage (Fig. 3C and D). Colitis category scores (inflammation, extent, regeneration and crypt damage) and overall colitis total scores (Fig. 3D) were all elevated in the Pos control animals. In the tryptophan-treated group, however, histological parameters were found to be at levels similar to the Neg group, suggesting a more rapid recovery from DSS-induced colitis in the animals supplemented with cysteine when compared to those in the Pos group.

The inflammatory and cellular turnover of the colon was also assessed by histological measurements of the mature and immature crypt cells and the muscle thickness. Crypt depth in Pos animals was significantly greater than that in animals in the Neg and Trp groups ($P<.001$) but had a mature crypt cell-to-immature crypt cell ratio similar to that in animals in the Neg group (Fig. 4A). This would indicate that the animals in the Pos and Neg groups had a similar ratio of mature to immature cells; however, the Pos group had a greater crypt depth. In contrast, the Trp group had reduced crypt depth and significantly lower mature crypt cell-to-immature crypt cell ratio than both the Neg and Pos groups ($P<.001$) (Fig. 4A and B). Furthermore, muscle thickness was significantly higher in the Pos group ($P<.001$) when compared to the Neg and Trp groups (Fig. 4C), indicating that tryptophan supplementation was able to ameliorate the DSS-induced colitis symptoms.

3.4. Measurement of colonic TNF-α and IL-6 by ELISA

TNF-α and IL-6 are critical pro-inflammatory cytokines associated with intestinal inflammation and damage in IBD. Concentrations of TNF-α and IL-6 in the colon were measured by ELISA as an indicator of the extent of local inflammation. Concentrations of both cytokines were elevated in the Pos group but significantly lower ($P<.01$ for IL-6 and $P<.05$ for TNF-α) in tryptophan-supplemented animals (Fig. 5). IL-6 and TNF-α levels were reduced by five- and fourfold, respectively, when compared to the Pos animals. No significant difference in concentrations of either cytokine was observed between untreated (Neg) animals and animals in the Trp group, indicating that tryptophan supplementation was able to reduce the DSS-induced inflammatory cytokine production to the basal levels seen in the Neg control animals.

3.5. Measurement of colonic mRNA expression by real-time RT-PCR

To further examine the effect of tryptophan supplementation on local immune responses, we examined colonic gene expression of several cytokines and inflammatory mediators involved in IBD pathogenesis. mRNA expression was measured by real-time quantitative RT-PCR. Results are expressed as relative mRNA expression levels and were calculated relative to porcine β-actin expression, which was stably expressed in all treatment groups. The expression of several cytokines with important immunoregulatory and pro-inflammatory functions was found to be elevated in animals in the Pos group and reduced with tryptophan supplementation. Relative mRNA levels of IL-6 ($P<.05$), TNF-α, IFN-γ ($P<.05$), IL-12p40 ($P<.01$), IL-1β ($P<.05$) and IL-17 were decreased in colon tissues of animals in the Trp group, to levels similar to those observed in the Neg control animals (Fig. 6A). Furthermore, mRNA expression of the chemokine IL-8 and intracellular adhesion molecule (ICAM)-1, which are involved in the characteristic neutrophil infiltration seen in IBD, was also significantly reduced ($P<.01$ and $P<.05$, respectively) upon tryptophan administration.

Analysis of genes involved in apoptosis revealed that the expression of the pro-apoptotic caspase-8 and Bax was significantly increased ($P<.001$ and $P<.01$, respectively) by treatment with tryptophan when compared to the Pos group (Fig. 6B). Expression

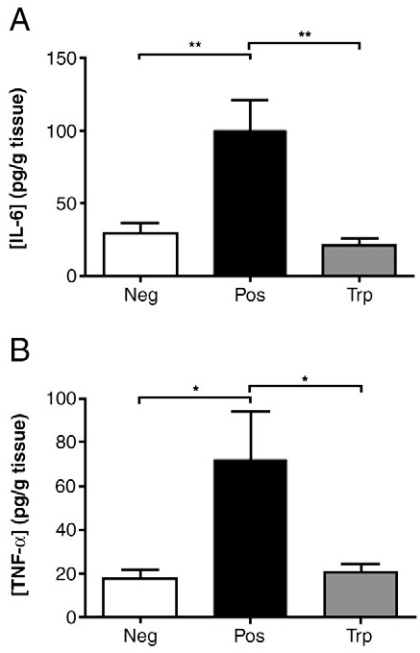

Fig. 5. Local colon concentrations of (A) IL-6 and (B) TNF-α per gram of tissue as measured by ELISA for Neg ($n=6$), Pos ($n=8$) and Trp ($n=7$). Values represent mean±S.E.M. $^{*}P<.05$; $^{**}P<.01$.

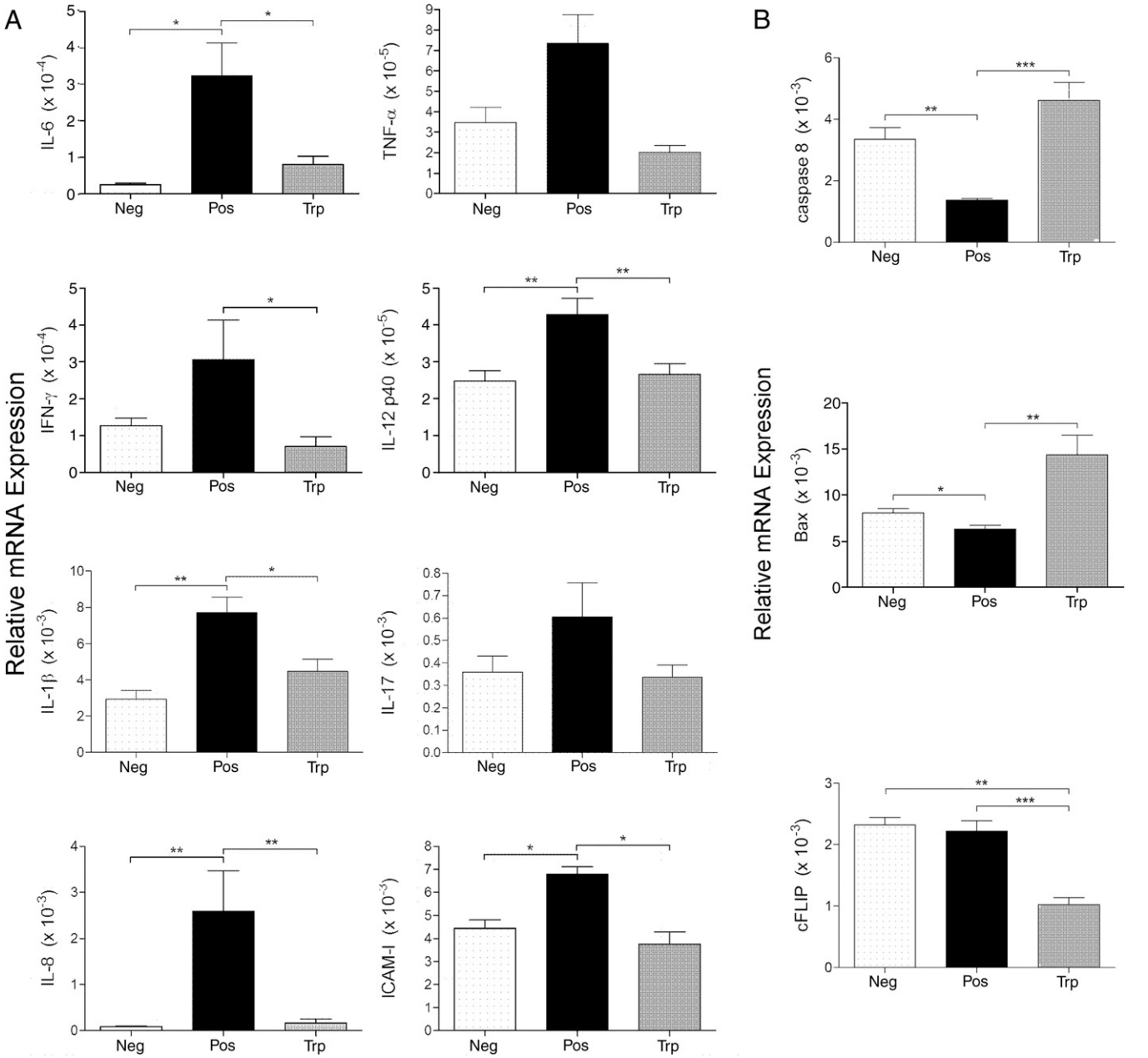

Fig. 6. Relative local gene expression in DSS-treated animals supplemented with tryptophan. Colon mRNA levels of (A) pro-inflammatory cytokines IL-6, TNF-α, IFN-γ, IL-12p40, IL-1β and IL-17 and cell recruitment and adhesion molecules IL-8 and ICAM-1 and (B) apoptosis mediators caspase-8, Bax and cFLIP were measured using real-time RT-PCR. Results are shown as relative mRNA expression of Neg (n=6), Pos (n=8) and Trp (n=7) groups using β-actin as the housekeeping gene. Values represent mean±S.E.M. $^{*}P<.05$; $^{**}P<.01$; $^{***}P<.001$.

of the anti-apoptotic or pro-survival gene cFLIP was significantly decreased ($P<.001$) by the administration of tryptophan.

4. Discussion

Although many treatment strategies for IBD have been proposed and clinically examined, additional therapeutic approaches are needed since many patients do not respond to the currently available options or exhibit significant side effects. As such, nutritional therapy, including the use of specific nutrients as primary treatment agents, has received considerable attention. In the present study, we examined the therapeutic effects of L-tryptophan in a porcine model of DSS-induced colitis. Tryptophan supplementation reduced colitis symptoms, improved histological parameters and intestinal permeability, reduced the expression of local inflammatory mediators and increased the expression of pro-apoptotic genes, important for maintaining gut homeostasis.

In humans, intestinal inflammation can cause weight loss due to increased metabolic rate, decreased dietary intake and malabsorbtion [32,33]. While the average feed intake of the Pos animals observed here was higher when compared to both the Neg and Trp groups, the BW gain to feed intake ratio of this group was significantly lower. This could be, in part, attributed to the increased gastrointestinal permeability observed in the Pos animals. Increased intestinal permeability and associated weight loss are common features in IBD and in DSS-treated animal models [8,34]. D-Mannitol, an inert small carbohydrate molecule that can be absorbed along the entire crypt–villus axis [8,29], was used to assess in vivo intestinal permeability. We observed that tryptophan administration reduced the in vivo gut

permeability when compared to animals in the Pos group. Recent literature shows that pro-inflammatory cytokines, such as IFN-γ and TNF-α, are able to alter the lipid and protein composition of the membrane microdomains of epithelial cell tight junctions, leading to damage in the mucosal barrier [35]. Here, tryptophan supplementation reduced the induction of the cytokines IFN-γ and TNF-α and, therefore, may have improved the membrane composition of the tight junctions and reduced gut permeability.

One of the major hallmarks of DSS-induced colitis is the profound colonic inflammation characterized by crypt destruction, mucosal ulceration, erosion and infiltration of lymphocytes into the mucosal tissue [36]. Indeed, this was observed in colon sections from Pos animals. However, reduced inflammation characterized by a reduction in cell infiltration and recovery of intact epithelium was evident in tryptophan-supplemented animals. Accordingly, the expression of IL-8, which contributes to IBD-mediated pathology via the recruitment of neutrophils to the intestinal mucosa [37], and ICAM-1 was significantly increased in the Pos group. ICAM-1 plays an important role in the recruitment of leukocytes to sites of inflammation and has been shown to be up-regulated in the intestinal mucosa of IBD patients [38,39]. Anti-ICAM-1 antibodies have been shown to be effective at reducing inflammation and attenuating colonic damage in animal models [40,41]. We observed a decrease in the expression of ICAM-1 in animals treated with tryptophan, suggesting that tryptophan supplementation may also aid in reducing the characteristic lymphocyte infiltration seen in IBD, leading to the resolution of inflammation and crypt damage observed here and facilitating healing of the colon. Upon cessation of DSS infusion, animals entered a recovery phase and began to repair the intestinal damage, during which there is an elongation of the colonic crypt in order to repair the mucosal damage by DSS [42]. Histological measurements revealed that crypt depth was greatest in the Pos group, although the mature crypt cell-to-immature crypt cell ratio was similar to that of the Neg group. Interestingly, the Trp group showed a reduction in the crypt depth and in the mature crypt cell-to-immature crypt cell ratios, indicating the ability of tryptophan to reduce the recovery period in tryptophan-supplemented animals. Furthermore, the smooth muscle thickening induced by DSS was ameliorated by tryptophan supplementation.

Cytokines play an important role in the initiation, modulation and amplification of the immune and inflammatory responses in IBD [5], and a number of therapies aimed at blocking their activity have been described [11]. Perhaps one of the most studied is TNF-α, one of the key pro-inflammatory cytokines involved in IBD pathogenesis [43,44]. TNF-α exerts its effects through a number of mechanisms, including the increased production of IL-6 and IL-1β, the increased expression of adhesion molecules and the inhibition of apoptosis [45]. Animals overexpressing TNF-α have been found to develop a Crohn's-like phenotype [46], and anti-TNF monoclonal antibodies have demonstrated therapeutic effects in IBD patients [47,48]. Although the precise mechanism of action of anti-TNF therapy is not fully understood, it has been shown to neutralize soluble TNF-α, alter cell signaling and induce the apoptosis of activated inflammatory cells. However, some patients are resistant to anti-TNF-α therapy and, therefore, may require therapies directed at other cytokines distinct from TNF-α [49]. In the present study, tryptophan supplementation reduced the DSS-induced local colon production of TNF-α and IL-6 at both the protein and gene expression levels, clearly demonstrating its anti-inflammatory and immune-modulating activity. Moreover, the expression of the pro-inflammatory mediators IFN-γ, IL-1β, IL-12p40 and IL-8, which have all been shown to be involved in IBD pathogenesis [48], was also significantly reduced. It has recently been determined that a novel subset of IL-17-producing lymphocytes, termed Th17 cells, may be critical in IBD pathogenesis [50]. The reduction in IL-17 expression observed here would suggest an additional mechanism by which tryptophan may ameliorate DSS-induced colitis symptoms and will require further study.

Tryptophan is intricately involved in the regulation of the immune system via the interaction between IFN-γ and the tryptophan-degrading enzyme IDO [18,51,52]. IDO possesses anti-inflammatory activity, suggested to occur via two mechanisms: the local depletion of tryptophan in surrounding immune cells and the anti-inflammatory activity of tryptophan metabolites [53]. The depletion of local tryptophan by activated antigen-presenting cells has been suggested to prevent T-cell proliferation and the release of pro-inflammatory cytokines [54,55], while tryptophan metabolites, referred to as kynurenines, can induce apoptosis of activated T cells [18]. The down-regulation of activated immune cells via apoptosis is a potent and effective strategy for controlling inflammatory reactions, and in patients with IBD, T-cell apoptosis has been found to be profoundly disturbed [56]. Indeed, we found that tryptophan supplementation decreased the expression of cFLIP, the porcine homologue of the Fas-associated-death domain-like IL-1-converting enzyme inhibitory protein (FLIP), which negatively regulates apoptosis by blocking caspase-8 activation [57]. Likewise, the expression of both caspase-8, an important initiator caspase in apoptosis, and Bax, a pro-apoptotic protein, was increased in tryptophan-supplemented animals and may have aided in restoring intestinal immune homeostasis.

We have demonstrated here that tryptophan may be an effective mucosal immune-modulating agent, inducing rapid recovery in tryptophan-supplemented animals via the down-regulation of inflammatory mediators and restoration of immune homeostasis in a DSS model of colitis. Further to its role in regulating mucosal inflammation, tryptophan supplementation may also aid patients in overcoming nutritional deficiency often observed in IBD, as well as acting as a substrate for the generation of serotonin, a mood-enhancing neurotransmitter that has also been shown to play a role in maintaining normal gut function [58]. Tryptophan is an essential nutrient found in many food products and has been successfully manufactured as an over-the-counter pharmaceutical. It is, therefore, a promising candidate for the treatment of IBD and, with additional clinical trials, may be a new treatment option for IBD patients.

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