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IDO1 Plays an Immunosuppressive Role in 2,4,6-Trinitrobenzene Sulfate–Induced Colitis in Mice

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IDO, an enzyme that degrades the essential amino acid L-tryptophan to N-formylkynurenine, is known to exert immunomodulatory effects in a number of diseases and disorders. IDO expression is increased in tumors, where it is thought to be involved in tumor evasion by suppressing the immune response. A competitive inhibitor of IDO is currently being tested in clinical trials for relapsed or refractory solid tumors; however, there remains a concern that attenuation of the immunosuppressive function of IDO might exacerbate inflammatory responses. In this study, we investigated the role of IDO in 2,4,6-trinitrobenzene sulfonate (TNBS)–induced colitis in mice by gene deletion and pharmacological inhibition. TNBS treatment induced significantly more severe colitis in Idol gene–deficient (Idol−/−) mice than in Idol wild-type (Idol+/+) mice, indicating a role for IDO1 in suppression of acute colitis. Consistent with this, the expression of Idol was increased in the colonic interstitial tissues of TNBS-treated Idol−/− mice. Furthermore, transplantation of Idol−/− bone marrow cells into Idol−/− mice reduced the pathological damage associated with colitis, altered the expression of cytokines, including IFN-γ, TNF-α, and IL-10, and increased the number of CD4+ Foxp3+ regulatory T cells in the colon. Pharmacological inhibition of IDO enzymatic activity by oral administration of 1-methyltryptophan (1-methyl-L-tryptophan or 1-methyl-D-tryptophan) significantly increased the severity of TNBS-induced colitis in mice, demonstrating that both stereoisomers can promote colitis. Collectively, our data indicate that IDO1 plays an important immunoregulatory role in the colon. The Journal of Immunology, 2013, 191: 000–000.

Indoleamine 2,3-dioxygenase (IDO) is an intracellular enzyme that catalyzes the degradation of the essential amino acid L-tryptophan to N-formylkynurenine, which is the first and rate-controlling step in the kynurenine pathway (1). IDO exerts immunosuppressive effects by reducing the local concentration of tryptophan and increasing the production of immunomodulatory tryptophan metabolites, which have a variety of effects on immune cells. For example, the metabolites suppress proliferation and promote apoptosis of T lymphocytes (2–4), and induce differentiation of naive T cells into regulatory T cells. In addition, recent studies have shown that increase of IDO concentrations in tissue inhibits migration of inflammatory cells, especially T cells (5). IDO is expressed in tissue macrophages and dendritic cells in a range of organs (6). Two types of IDO are known: IDO1 and the recently identified IDO2 (7). Although the two enzymes have a similar range of substrates, they show different tissue distributions (8). In both humans and mice, IDO1 is widely distributed, including in the gut (distal ileum and colon), lymph nodes, spleen, thymus, and lungs, whereas IDO2 expression is limited to the kidney, liver, and reproductive system (8). Tryptophan dioxygenase also catalyzes tryptophan breakdown; however, this enzyme is expressed almost exclusively in the liver and is thought to have no influence on the immune system (9, 10).

IDO has attracted considerable attention as a novel target for the development of cancer therapeutics. Some tumors express higher IDO levels than do normal tissues, which is thought to contribute to their escape from attack by the host immune system (11). Treatment with an IDO inhibitor has also been reported to reduce the volume of tumors transplanted into mice preimmunized with the tumor Ag (12). These data suggest that competitive inhibitors of IDO, such as 1-methyltryptophan (1-mT), may mediate antitumor effects by suppressing immune tolerance. 1-mT is currently in clinical trials for treatment of some recurrent solid tumors (13). Given the immunosuppressive role of IDO, however, there is concern that IDO inhibitors could exacerbate inflammatory reactions, which would be particularly harmful for patients with inflammatory disorders. Although colon cancer is a potential candidate for treatment with IDO inhibitors (14), a previous study has shown that 1-mT augments 2,4,6-trinitrobenzene sulfonic acid (TNBS)–induced colitis in mice (15). 1-mT exists as two stereoisomers, 1-methyl-D-tryptophan (1-D-mT) and 1-methyl-L-tryptophan (1-L-mT), and recent studies have shown that 1-L-mT preferentially inhibits IDO1, whereas 1-D-mT not only inhibits IDO2 (7), but also increases IDO1 expression (13). Because most preclinical studies have used the racemic mixture 1-D-1-L-mT, different approaches to inhibiting IDO are necessary to elucidate the role of the enzyme in colitis.

In this study, we have investigated the immunomodulatory function of IDO1 in chemically induced colitis by using Idol−/− mice.
mice and a competitive inhibitor of IDO, 1-mT. We examined the effects of 1-1-mT and 1-0-mT separately to elucidate the differences between the stereoisomers. This study is of particular relevance for understanding the efficacy and safety of IDO inhibition in clinical trials.

Materials and Methods

Animals

Ido1<sup>-/-</sup> mice of the C57BL/6 background were obtained from The Jackson Laboratory (Bar Harbor, ME). Ido1<sup>-/-</sup> mice were crossed with C57BL/6J (Charles River Japan, Yokohama, Japan) to generate the Ido1<sup>-/-</sup> and Ido1<sup>+/+</sup> offspring used in this study. GFP transgenic (Tg) mice were obtained from SLC (Wilmington, MA). Mice were housed in plastic cages (four to five mice/cage) under controlled conditions of light (12-h light/dark cycle) and temperature (23 ± 2°C). All animals were handled in accordance with the regulations for animal experiments at Gifu University.

Induction of acute colitis

Eight-week-old Ido1<sup>-/-</sup> or Ido1<sup>+/+</sup> mice were assigned to three groups: TNBS-treated, ethanol-treated (TNBS solvent control), and untreated controls. Group sizes are indicated in the figures. Treatment of the mice with ethanol and TNBS (purchased from Sigma-Aldrich, St. Louis, MO) was performed as described previously (16). In brief, mice were placed under anesthesia and 100 μl TNBS (2.5% TNBS in 50% ethanol) or 50% ethanol was instilled intrarectally. All surviving mice were sacrificed 3 d later.

Bone marrow transplantation

Five-week-old Ido1<sup>-/-</sup> or Ido1<sup>+/+</sup> mice were transplanted with Ido1<sup>-/-</sup> or Ido1<sup>+/+</sup> GFP-expressing bone marrow cells (BMCs). To prepare donor BMCs, Ido1<sup>-/-</sup> and Ido1<sup>+/+</sup> mice were crossed with GFP-Tg mice, which constitutively express GFP under the control of the CAG promoter, to generate Ido1<sup>-/-</sup>:GFP-Tg or Ido1<sup>+/+</sup>:GFP-Tg mice (F2). Bone marrow transplantation was performed as described previously (17). All recipients were given 500 U/ml gentamicin sulfate (Invitrogen, Grand Island, NY) in the drinking water starting 2 d before and continuing for 26 d after transplantation. On day 0, the recipient mice were irradiated in a split dose (5.0 Gy twice with a 4-h interval) and injected with unfractionated BMCs (5 × 10<sup>6</sup> in 250 μl RPMI 1640 medium; Sigma-Aldrich) via the tail vein. Gentamicin sulfate was withdrawn on day 26 posttransplantation, and 2 d later, the mice were treated with TNBS as described earlier (experimental protocol is given in Supplemental Fig. 1). Three days later, the mice were sacrificed and analyzed. Bone marrow was examined for cellularity and GFP<sup>+</sup> cells to confirm successful engraftment of the donor cells.

1-mT treatment

Eight-week-old Ido1<sup>+/+</sup> mice were assigned to three groups: 1-1-mT treatment, 1-0-mT treatment, and vehicle treatment (control). In addition, a group of Ido1<sup>-/-</sup> mice of the same age was treated with 1-1-mT to verify the off-target effects of the IDO inhibitor. Throughout the experimental period, the mice were administered 1-1-mT or 1-0-mT (both purchased from Sigma-Aldrich) in the drinking water (5 mg/ml, pH 10.7) or alkaline water adjusted to pH 10.7 (control group). On day 4 of the experiment, all mice received TNBS treatment as described earlier. The surviving mice were sacrificed on day 7.

Sample preparation

The colon was opened longitudinally and cut in half in the direction of the long axis. One half was fixed in 10% buffered formalin for 24 h at room temperature and processed for histological and immunohistochemical analyses as described later. The second half of the colon was processed for quantitative real-time RT-PCR analysis and enzymatic assays.

Histological analysis

Paraffin-embedded sections (3 μm thick) of the colon were prepared and stained with H&E for histological grading. The colon tissue was divided into 16 separate sections. The histological grades were determined for each section and the sum of the grades was reported as the inflammation score for each mouse. Histological grading of colitis was determined on a scale of 0 to 5, as described in other studies (15, 18), with some modifications.

Grade 0: no obvious inflammation; Grade 1: mild inflammatory cell infiltration, no structural changes observed; Grade 2: moderate inflammatory cell infiltration, crypt elongation, bowel wall thickening that does not extend beyond the mucosal layer, no evidence of ulceration; Grade 3: severe inflammatory cell infiltration, thickening of bowel wall, high vascular density, crypt elongation with distortion, transmural bowel wall thickening with ulceration; Grade 4: complete loss of mucosal architecture (crypts) with ulceration and loss of mucosal vasculature; and Grade 5: coagulation necrosis of at least the mucosal layer. Representative images of colons at each grade are shown in Supplemental Fig. 2.

Immunohistochemical analysis

Immunohistochemical analyses were performed on paraffin-embedded colon sections. The primary Abs were rabbit anti-IDO (a gift from K. Saito, Kyoto University), rat anti-F4/80 (clone AA3-1; AbD Serotec, Kidlington, U.K.), rabbit anti-CD4 (Abcam, Cambridge, U.K.), rabbit anti-FoxP3 (Abcam), rabbit anti-GFP (Abcam), and Armenian hamster anti-CD11c (clone N418; Abcam). After deparaffinization and rehydration, all sections except those used for anti-CD4 staining were soaked in 3% hydrogen peroxide in methanol for 10 min to remove endogenous peroxidase activity. Ag retrieval was performed with 0.01 M citrate buffer (pH 6.0) at 121°C. Sections were then blocked with 2% BSA in PBS to reduce nonspecific staining and incubated with primary Abs overnight at 4°C. Biotinylated secondary Abs (Dako, Glostrup, Denmark) were then added for 30 min at room temperature. Signal amplification was performed with avidin-biotin-peroxidase complex (Vectastain; Vector Laboratories, Burlingame, CA), followed by a chromogenic reaction with 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich). The sections were then counterstained with hematoxylin. For CD11c and IDO double staining, the sections were incubated with primary Abs overnight at 4°C. Biotinylated anti-Armenian hamster Ab (Abcam) and HRP-labeled anti-rabbit Ab (Dako) were then added for 30 min at room temperature. Signal amplification was performed with alkaline phosphatase-biotin complex (Vectastain) followed by a chromogenic reaction with alkaline phosphatase substrate kit (VectaBlue; Vector Laboratories) and 3,3′-diaminobenzidine tetrahydrochloride. The sections were then counterstained with methyl green.

![FIGURE 1. Effects of IDO1 deficiency on TNBS-induced colitis. (A) Histological inflammation scores for colons of TNBS-treated, ethanol-treated, and untreated mice. The TNBS- and ethanol-treated Ido1<sup>-/-</sup> mice developed significantly more severe colitis than the equivalent groups of Ido1<sup>+/+</sup> mice. Data are mean ± SD of the indicated number of animals. In both genotypes, the histological scores were significantly higher in TNBS-treated mice than in untreated mice. *p < 0.05, **p < 0.001 by Student t test. (B) Representative images of H&E-stained colons from each group of mice. Note that the most severe colitis with coagulation necrosis is in TNBS-treated Ido1<sup>-/-</sup> mice. Scale bars, 150 μm.](http://www.jimmunol.org/doi/abs/10.4049/jimmunol.1700699)
Crypt isolation
The second half of the colon tissue was used to isolate epithelial crypts from the interstitium. For this, the tissue was incubated in HBSS (Sigma-Aldrich) containing 30 mM EDTA for 15 min at 37°C and then vortexed vigorously in HBSS without EDTA.

Quantitative real-time RT-PCR
Isolated epithelial crypts and interstitial tissues were homogenized (Bullet Blender Storm; Chiyoda Science, Tokyo, Japan), and total RNA was extracted using an RNeasy Mini kit (Qiagen, Tokyo, Japan). cDNA was synthesized using SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, Tokyo, Japan) according to the manufacturer’s instructions. Quantitative real-time RT-PCR of eight genes of interest (Fabp2, Fsp1, Ido1, Ido2, Il-10, and Tgfb) was performed using SYBR Premix Ex Taq (Takara Bio, Shiga, Japan) on a Thermal Cycler Dice Real Time System (Takara Bio), essentially as previously described (19). The PCR primers are listed in Supplemental Table I. The expression of each gene was normalized to β-actin (Actb) expression by using the standard curve method.

IDO enzyme assay
IDO activity was assayed as described previously (20). In brief, tissues were homogenized with a Polytron homogenizer (Kinematica, Bohemia, NY) in 1.5 vol ice-cold buffer (140 mM KCl and 20 mM potassium phosphate buffer, pH 7.0) and then centrifuged at 7000 × g for 10 min at 4°C. An aliquot of 50 μl supernatant was mixed with 50 μl substrate solution for the measurement of IDO activity. The substrate solution consisted of 100 mM potassium phosphate buffer (pH 6.5), 50 μM methylene blue, 20 μg catalase, 50 μM ascorbate, and 400 μM L-tryptophan. The reaction mixtures were incubated for 60 min at 37°C, acidified with 3% perchloric acid, and then centrifuged at 7000 × g for 10 min at 4°C. The concentrations of the enzymatic products were measured by HPLC as described previously (20). IDO enzyme activity was expressed as the amount of product generated per hour incubation per gram of tissue protein.

Statistical analysis
For comparisons of the two groups, the data were analyzed using the unpaired Student t test with Welch’s correction, except for the analysis of Fpob2 and Fsp1 expression (Supplemental Fig. 3A, 3B), which was performed using the paired Student t test with Welch’s correction. For comparisons of more than two groups, we used the Kruskal–Wallis test followed by Dunn’s test for multiple comparisons or followed by Steel’s test for comparison with controls. A p value <0.05 was considered statistically significant.

Results
IDO1 deficiency exacerbates TNBS-induced colitis
To investigate the role of IDO1 in experimental colitis, we examined colon histology in Ido1−/− and Ido1+/+ mice treated with TNBS. In mice of both genotypes, the histological grades of colonic inflammation were consistently higher in TNBS-treated mice than in ethanol-treated and untreated mice (Fig. 1A, 1B).
addition, Idol−/− mice developed significantly more severe colitis than did Idol+/+ mice (p < 0.001; Fig. 1A, 1B), demonstrating that IDO1 deficiency exacerbates TNBS-induced colonic inflammation. TNBS treatment induced severe transmural inflammation frequently associated with changes in mucosal architecture including extensive ulceration and coagulation necrosis in the colon of Idol−/− mice (Fig. 1B). In contrast, Idol+/+ mice had relatively mild inflammation and occasionally developed focal ulceration (Fig. 1B). Similarly, Idol−/− mice treated with ethanol had significantly more severe colitis than Idol+/+ mice (p < 0.05; Fig. 1A). These observations indicate that IDO1 suppresses chemically induced colitis in mice.

IDO expression increases with exacerbation of colonic inflammation

Immunohistochemical staining of colons revealed that IDO was expressed at the sites of inflammation in Idol+/+ mice treated with TNBS (Fig. 2A). Strong IDO expression was observed in inflammatory cells, including CD11c+ dendritic cells, in the lamina propria and submucosa (Fig. 2A, right). Idol and Idod expression in isolated epithelial and interstitial tissues was quantified by RT-PCR. The purity of the fractions was first confirmed by analyzing the epithelial marker Fabp2 and mesenchymal marker Fsp1, as described previously (19). As expected, Fabp2 expression was significantly higher in the epithelial tissue, whereas Fsp1 was preferentially expressed in the interstitial tissue (p < 0.0001; Supplemental Fig. 3A, 3B). As shown in Fig. 2B, the expression of Idol was the highest in both interstitial and epithelial colon tissues of TNBS-administered Idol+/+ mice, with a significant difference being detected in interstitial tissues (p < 0.05; Fig. 2B). Idol expression in the interstitial tissues of the ethanol-treated animals was intermediate between that of the TNBS-treated and control groups. These results indicate that IDO1 expression increased preferentially in interstitial tissues during colonic inflammation. Unlike IDO1, IDO2 is not strongly expressed in normal colon tissues (8). Nevertheless, Idod expression was upregulated by TNBS or ethanol treatment, and there was a significant difference between the TNBS-treated group and the control group (p < 0.05; Fig. 2B), suggesting that this enzyme could play a role in the inflammation. Consistent with the increase in mRNA and protein expression, IDO enzyme activity also increased in TNBS-treated Idol+/+ mice, although the differences were not significant (Fig. 2C). Because IDO1 is rapidly induced by IFN-γ in dendritic cells (21), we examined the expression of Ifng in the interstitial tissues. In Idol+/+ mice, Ifng expression was increased by TNBS treatment (Supplemental Fig. 3C). In addition, Ifng expression was markedly increased in ethanol- and TNBS-treated Idol−/− mice, reflecting the feedback relationship between IDO and IFN-γ (22) (Supplemental Fig. 3C).

We found that IDO expression and activity in epithelial tissues were also increased by TNBS treatment (Fig. 2B, 2C). This finding suggests that IDO expressed in epithelial cells might also contribute to the regulation of colonic inflammation.

Transplantation of Idol+/+ BMCs attenuates TNBS-induced colitis in Idol−/− mice

To determine whether the transfer of IDO1-expressing hematopoietic cells could reduce TNBS-induced colitis, we transplanted GFP-labeled Idol−/− or Idol+/+ BMCs in Idol−/− or Idol+/+ mice, and 4 wk later, the mice were treated with TNBS (Supplemental Fig. 1). On day 3, the animals were sacrificed for the analysis of colons. Bone marrow samples were also examined to confirm engraftment of donor cells, and no difference in cellularity was observed among the groups (data not shown). Immunohistochemical analysis of GFP+ cells revealed that donor bone marrow–derived cells, including lymphocytes, neutrophils, macrophages, and dendritic cells, had migrated into the colon wall where they were scattered within the stromal tissue (Fig. 3A). In keeping with the high susceptibility of Idol−/− mice to TNBS, Idol−/− mice transplanted with Idol+/+ BMCs had the most severe colitis (Fig. 3B). Importantly, transplantation of Idol+/+ BMCs into the Idol−/− mice reduced the colitis severity significantly (p < 0.05; Fig. 3B), demonstrating that the Idol+/+ BMCs were able to attenuate the TNBS-induced colitis in Idol−/− mice. In contrast, Idol+/+ mice developed more severe colitis when transplanted with Idol−/− BMCs than with Idol+/+ BMCs. These results indicate that bone marrow–derived inflammatory cells would play central roles in the suppression of acute inflammation by expressing IDO1.

The results shown in Fig. 3 indicate that the severity of TNBS-induced colitis was also influenced by the genotype of the transplant recipient mice. When Idol−/− BMCs were transplanted, TNBS induced more severe colitis in Idol−/− recipient mice than in Idol+/+ recipient mice. Similarly, when Idol+/+ BMCs were transplanted,
Ido1−/− mice exhibited more severe colitis than did the Ido1+/+ recipients. These findings suggest that colonic epithelial cells may play a role in regulating acute colitis in mice.

Inflammatory cytokine production in colons is affected by IDO1 deficiency

To examine the effect of IDO1 deficiency on the production of proinflammatory cytokines at the site of TNBS-induced inflammation, we examined the expression levels of IFN-γ and TNF-α by quantitative RT-PCR after TNBS treatment of transplanted mice. Expression of Ifng and Tnf in the colons of Ido1−/− mice was higher after reconstitution with Ido1−/− BMCs than with Ido1+/+ BMCs (Fig. 4). Conversely, the expression of these cytokines in Ido1+/+ mice was higher when the mice were reconstituted with Ido1−/− BMCs than with Ido1+/+ BMCs (Fig. 4). We also examined the expression of the anti-inflammatory cytokines IL-10 and TGF-β to elucidate the immunoregulatory function of IDO1. In both Ido1−/− and Ido1+/+ mice, expression of Il-10 in the colons was significantly higher after reconstitution with Ido1+/+ BMCs than with Ido1−/− BMCs. This result indicates the importance of Ido1+/+ BMCs in regulating inflammation through the induction of anti-inflammatory cytokines. The expression of Tgfb was slightly higher in Ido1−/− mice reconstituted with Ido1+/+ BMCs than with Ido1−/− BMCs, but this was not significantly different. Taken together, these data suggest that BMC-derived, IDO1-expressing inflammatory cells regulate chemically induced colitis through the expression of proinflammatory and anti-inflammatory cytokines.

IDO1 deficiency results in the decrease of regulatory T cells

IDO-expressing dendritic cells have been shown to increase the differentiation of naive CD4+ T cells into regulatory T cells (23).

To investigate the effect of IDO1 deficiency on T cell differentiation at the site of TNBS-induced inflammation, we quantified regulatory T cells by immunohistochemical staining of colon samples with Abs against Foxp3, a transcription factor specifically expressed by regulatory T cells. Total CD4+ T cell and Foxp3+ regulatory T cells were enumerated on sequential sections of colons (Fig. 5A), and the ratio of regulatory T cells to total CD4+ T cells (Foxp3/CD4 ratio) was calculated. A significantly lower Foxp3/CD4 ratio was detected in the colons of Ido1−/− mice reconstituted with Ido1−/− BMCs than with Ido1+/+ BMCs (p < 0.05; Fig. 5B), indicating that the IDO1 expression was necessary for naive CD4+ cells to differentiate into Foxp3+ regulatory T cells. Interestingly, the transplantation of Ido1−/− BMCs into Ido1+/+ recipient mice also significantly decreased the Foxp3/CD4 ratio in the colonic tissue (p < 0.05; Fig. 5B), despite the expression of IDO1 in the recipient epithelial tissues. These results suggest that IDO1 expression in bone marrow–derived inflammatory cells is necessary to promote the differentiation of regulatory T cells in the colon.

Inhibition of IDO by 1-L-mT or 1-D-mT has different effects on colitis

To evaluate the effects of IDO inhibitors in chemically induced colitis, we treated Ido1+/+ mice with TNBS and administered either 1-L-mT or 1-D-mT. The inhibitor-treated mice developed significantly more severe colitis than control mice (Fig. 6), demonstrating that both stereoisomers promote colitis. Ido1−/− mice treated with TNBS were also administered 1-L-mT to evaluate the off-target effects. The histological grades of Ido1−/− and Ido1+/+ mice treated with 1-L-mT were equivalent (Fig. 6), confirming that 1-L-mT preferentially inhibits IDO1, as previously reported (7), and exacerbates colitis. Interestingly, the colonic inflammation...
grades of mice treated with 1-o-mT were also higher than the grades of control mice with a significant difference. Given that 1-o-mT preferentially inhibits IDO2 (24), and Id2 expression was significantly upregulated in the colon of TNBS-treated mice (Fig. 2B), this result indicates that IDO2 inhibition can have a substantial effect on chemically induced colitis.

**Discussion**

IDO function has been studied in a variety of diseases, including viral infections, autoimmune diseases, and cancer. Although the gastrointestinal tract has been shown to express abundant IDO activity, suggesting a possible role for this enzyme in intestinal homeostasis and disease, relatively little is known of the function of IDO in intestinal disorders. In the mouse gut, IDO expression is the highest in the small intestine and is ~10-fold lower in the cecum and colon (10). Previous studies have shown that IDO expression is elevated in a number of intestinal inflammatory conditions, including ulcerative colitis, Crohn’s disease, and celiac disease (25, 26), suggesting a need for further investigation of the role of IDO in intestinal inflammation. In this regard, Gurtner et al. (15) showed that the IDO inhibitor 1-mT significantly increased the severity of TNBS-induced colitis in mice. In this study, to precisely investigate the function of IDO1, this enzyme predominantly expressed in the intestine, we took a different approach by using an Ido1−/− model. We showed that IDO1 deficiency markedly exacerbates TNBS-induced colitis, demonstrating a key role for this enzyme in the suppression of acute inflammation in the colon.

Furthermore, consistent with previous studies demonstrating that IDO1 promotes the differentiation and migration of regulatory T cells (23, 27), IDO1 deficiency significantly decreased Foxp3+ regulatory T cells at the inflammatory site, which is thought to be involved in exacerbation of TNBS-induced colitis. Boehm et al. (28) recently demonstrated that deletion of Foxp3+ regulatory T cells worsens acute intestinal inflammation with significantly increased cytokine production including IFN-γ.

The main source of IDO production in the intestines remains controversial. Some studies have noted that colonic IDO appears to be expressed largely in the epithelium (10), whereas other studies indicated that IDO is mainly expressed in local dendritic cells in the intestinal lamina propria (6). In this study, we showed that Ido1 mRNA was expressed at similar levels in the colonic epithelial and interstitial tissues of untreated wild-type mice, supporting the earlier findings that colonic epithelium expresses IDO under physiological conditions (10). However, we found that Ido1 expression was increased predominantly in the interstitial tissues of mice treated with TNBS. Consistent with this, the IDO protein was detected at high levels in the inflammatory cells in the lamina propria and submucosa of the colon in TNBS-treated mice. The results of our BMC transplantation experiments also support a limited role for IDO expressed in colonic epithelial cells. Transplantation of Ido1−/− BMCs increased the severity of TNBS-induced colitis in Ido1+/+ recipient mice (Fig. 3B), despite the fact that the epithelial cells of these mice maintained the ability to express IDO1. Nevertheless, the colitis was still milder in Ido1+/+ mice reconstituted with Ido1+/− BMCs than in Ido1−/− mice reconstituted with Ido1+/− BMCs, suggesting that IDO1 expression in the colonic epithelium does contribute to the suppression of inflammation.

In this study, we investigated the effect of the IDO inhibitor 1-mT on TNBS-induced colitis, focusing on the differences between the stereoisomers. Recent studies have shown that the L and D stereoisomers of 1-mT show differential inhibition of IDO (7, 24). However, most preclinical studies, including colitis models, have used the racemic mixture of 1-D/L-mT, and the individual effects of the isomers on colon tissues have not been well documented. 1-l-mT has been reported to preferentially inhibit IDO1 (7). Accordingly, we found that the phenotype of Ido1+/+ mice treated with 1-l-mT was similar to that of Ido1−/− mice (Figs. 1, 6). We
observed no off-target effects of 1-L-mT in Idod1−/− mice (Fig. 6), demonstrating that 1-L-mT exacerbates colonic inflammation by inhibiting IDO1. In contrast, 1-D-mT has a greater inhibitory effect on IDO2 than on IDO1 (24). Interestingly, Idod1−/− mice treated with 1-D-mT developed significantly more severe colitis than control mice, suggesting that IDO2 could also play an important role in colitis. This possibility is supported by the increased expression of Idod2 in the colon of TNBS-treated mice (Fig. 2B). Because IDO2 is expressed at lower levels than IDO1 in normal colon tissue (8), IDO2 is unlikely to be involved in the maintenance of normal tissue homeostasis but may be readily induced in parallel with IDO1 under inflammatory conditions. However, unlike Idod1 expression levels, the levels of Idod2 expression in intestinal tissues were higher in the ethanol-treated mice than in the TNBS-treated mice, suggesting that IDO2 is not necessarily increased with the exacerbation of colitis.

IDO is expressed at high levels in some solid tumors, including colorectal cancer, where it is thought to play a role in immune evasion (9, 29–32). Consistent with this, the competitive IDO inhibitor 1-mT is currently in clinical trials for the treatment of relapsed or refractory solid tumors (13, 33). Considering that the 1-mT form used in clinical trials is actually the 1-D-mT stereoisomer (13), it is particularly noteworthy that 1-D-mT exacerbated TNBS-induced colitis. Colorectal cancer may also be a candidate for treatment with IDO inhibitors. However, our data indicate that IDO inhibition can exacerbate inflammation in the colon, suggesting that particular care should be taken when treating patients who have autoimmune or inflammatory diseases such as Crohn’s disease or ulcerative colitis.

Recent studies revealed that IDO plays a role in intracellular signaling in dendritic cells and contributes to TGF-β-driven tolerance in noninflammatory conditions (34–36). In this study, signaling in dendritic cells and contributes to TGF-β-driven tolerance in noninflammatory conditions.

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Disclosures
The authors have no financial conflicts of interest.

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