Dietary tryptophan alleviates dextran sodium sulfate-induced colitis through aryl hydrocarbon receptor in mice

Jahidul Islam, Shoko Sato, Kouichi Watanabe, Takaya Watanabe, Ardiansyah, Keisuke Hirahara, Yukihide Aoyama, Shuhei Tomita, Hisashi Aso, Michio Komai, Hitoshi Shirakawa

*Laboratory of Nutrition, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan
1Department of Biological Science and Technology, Tokyo University of Science, Tokyo, Japan
2Cellular Biology Laboratory, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan
3International Education and Research Center for Food Agricultural Immunology, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan
4Department of Food Science and Technology, Universitas Baktie, Jakarta, Indonesia
5Department of Pharmacoogy, Graduate School of Medicine, Osaka City University, Osaka, Japan

Abstract

Ulcerative colitis is the typical progression of chronic inflammatory bowel disease. Amino acids, particularly tryptophan, have been reported to exert a protective effect against colitis induced by dextran sodium sulfate (DSS), but the precise underlying mechanisms remain incompletely clarified. Tryptophan metabolites are recognized to function as endogenous ligands for aryl hydrocarbon receptor (Ahr), which is a critical regulator of inflammation and immunity. Thus, we conducted this study to investigate whether dietary tryptophan supplementation protects against DSS-induced colitis by acting through Ahr. Female wild-type (WT) and Ahr−deficient (knockout; KO) mice (10–12 weeks old) were divided into four groups and fed either a control or 0.5% tryptophan diet. The tryptophan diet ameliorated DSS-induced colitis symptoms and severity in WT mice but not in KO mice, and the diet reduced the mRNA expression of Il-6, Tgfβ, Il-1β and the chemokines Ccl2, Cxcl1 and Cccl2 in the WT groups. Furthermore, Il-22 and Stat3 mRNA expression in the colon was elevated in WT mice fed with the tryptophan diet, which mainly protected epithelial layer integrity, and Ahr also modulated immune homeostasis by regulating Foxp3 and Il-17 mRNA expression. These data suggest that tryptophan-containing diet might ameliorate DSS-induced acute colitis and regulate epithelial homeostasis through Ahr. Thus, tryptophan could serve as a promising preventive agent in the treatment of ulcerative colitis.

© 2017 Elsevier Inc. All rights reserved.

Keywords: Aryl hydrocarbon receptor; Dextran sodium sulfate; Tryptamine; Tryptophan; Ulcerative colitis

1. Introduction

Ulcerative colitis (UC) is the typical progression of chronic inflammatory bowel disease (IBD) [1]. IBD is a fatal and relapsing inflammatory disorder of the gastrointestinal (GI) tract that is characterized by chronic, uncontrolled inflammation in the intestinal mucosa, and IBD affects millions of people worldwide and incurs a substantial economic cost [2]. The severity and pathogenesis of UC are considered to be influenced by multiple genetic factors, immune responses, intestinal flora and oxidative stress [3], and the conventional UC treatments used to date have demonstrated variable efficacy, adverse side effects and potential long-term toxicity [4,5]. Therefore, it is critical to develop alternative beneficial strategies for UC treatment.

Tryptophan is an essential amino acid for mammals that is readily obtained through the diet and is regarded as a key regulator of metabolic pathways, and recent findings have demonstrated that tryptophan plays a role in protecting health [1,6–8]. In the GI tract, tryptophan can enter several metabolic pathways, most notably protein synthesis, serotonin, and kynurenine pathways and microbiota-mediated degradation [9,10]. Tryptophan metabolites such as indole-3-aldehyde, kynurenine, indole-3-acetic acid and tryptamine can act as ligands for aryl hydrocarbon receptor (Ahr) [10–13], which is a ligand-activated transcription factor and a member of the basic helix-loop-helix/Per–Arnt–Sim homology superfamily [14]. Ahr functions as a cytosolic sensor of xenobiotics such as polycyclic aromatic compounds, including 2,3,7,8-tetrachlorodibenzo-p-dioxin [15]. Upon ligand binding, Ahr translocates from the cytoplasm into the nucleus, where it heterodimerizes with Ahr nuclear translocator (Arnt) and then activates the transcription of several genes involved in xenobiotic metabolism, including members of the cytochrome P450 superfamily [14–16]. Ahr mediates several toxic
effects, including endocrine disruption, tumor development, cell differentiation, thymic atrophy and immune suppression, and as compared to wild-type (WT) mice, Ahr-deficient (knockout; KO) mice are more susceptible to toxicological effects, including advancement of cardiac hypertrophy, impairment of embryonic development, skin lesions and abnormal liver development [17,24]. Ahr regulation by its natural ligands has been shown to lead to an abolishment carcinogenesis in the mouse intestine, where Ahr functions as a critical player in mucosal barrier defense, most notably because of its role in interleukin (IL)-22 production by innate lymphoid cells (ILCs) [15,18]. IL-22 also serves as a strong activator of the pleiotropic transcription factor Stat3 (signal transducer and activator of transcription 3) [19]. Moreover, Ahr controls the regulatory T cells (Tregs) that express the forkhead transcription factor Foxp3, which play a critical role in immune tolerance [20,21].

Ahr has been reported to play a key role in attenuating UC induced by dextran sodium sulfate (DSS) [22]. DSS carries a highly negative charge that is contributed by sulfate groups, and DSS is toxic to colonic epithelia and induces erosions; this ultimately results in an abnormally inflamed microenvironment and the activation of inflammatory cells and enhancement of inflammatory mediators, leading to severe colitis. DSS-induced colitis exhibits several characteristics resembling those of human UC [23]. A few previous studies have described a role of tryptophan supplementation in protection against UC [1,10], but the precise molecular mechanisms involved remain incompletely understood. Here, we investigated the functional involvement of Ahr in intestinal inflammation by providing a tryptophan-containing diet to mice and then examined the regulatory contribution of IL-22/Stat3. Our main objective in this study was to elucidate the potential mechanisms by which dietary tryptophan supplementation protects against DSS-induced colitis through Ahr.

2. Materials and methods

2.1. Materials

We purchased DSS salt (MW = 40 kD) from Sigma-Aldrich (St. Louis, MO, USA), tryptophan from Wako Pure Chemicals (Tokyo, Japan), and myeloperoxidase (MPO) from Biovision (Milpitas, CA, USA). The amounts of tumor necrosis factor α (TNFα) and IL-6 in mouse serum were determined using commercially available mouse enzyme-linked immunosorbent assay (ELISA) kits from Diacline SAS (Besancon Cedex, France) and R&D Inc. (Minneapolis, MN, USA), respectively.

2.2. Animals

Female C57BL/6 WT and KO mice (10–12 weeks old) were used in experiments and all mice were bred and maintained in littermates. The KO mice were kindly provided by Dr. Frank J. Gonzales [24]. Mice were housed in plastic cages (3–4 mice per cage) containing paper-chip bedding, under controlled temperature (23 ± 2 °C), 50% ± 10 humidity and a 12:12-h preset light–dark cycle. The Animal Research–Animal Care Committee of Tohoku University approved the experimental plan of this study. All experiments were conducted under the guidelines issued by this committee in accordance with Japanese governmental legislation (2005).

2.3. Experimental design

Mice were provided a control diet (AIN93M standard diet for rodents) or the control diet supplemented with 0.5% tryptophan (experimental diet) [1]. Composition of diets was illustrated in Supplementary Table 1. To investigate the functional interaction between the tryptophan diet and Ahr, mice were allocated to four groups: control diet, WT and KO groups (n=8 each). The mice received these diets starting at 4 days before the initial DSS administration; after 4 days, fresh feces were collected for determining fecal tryptamine levels [25]. On the day of DSS administration (Day 0), initial body weight was measured, and DSS colitis was induced in all groups by including 3.5% DSS in drinking water for 8 days. Consumption of the DSS solution, food intake and body weight of the animals were monitored and recorded daily. The disease activity index (DAI) was obtained based on a combination of stool consistency and fecal bleeding, as described by Nishiyama et al. [26]. Briefly, the DAI was calculated by adding the Diarrheal Score (0, normal stool; 1, mild soft stool; 2, very soft stool; 3, watery stool), with the score for the presence or absence of fecal blood (0, normal; 2, brown stool; 3, reddish stool; 4, bloody stool). After 8 days, mice were sacrificed, and their serum, colon

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>TrpX</td>
<td>5′-GACCTGCGAATCTGAGCACAAGG-3′</td>
<td>5′-TCTCTGATGGCCCCCTATTCTC-3′</td>
</tr>
<tr>
<td>IL-1β</td>
<td>5′-CTTTGTCCTTCCCTTGGGCAC-3′</td>
<td>5′-CAGCTCATATGTTGCTGCA-3′</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>5′-GCAAGCCACCTGACGAG-3′</td>
<td>5′-ATAACCACATTTAGCTGCA-3′</td>
</tr>
<tr>
<td>Tnfα</td>
<td>5′-AGTTGGCTCAGCCAGATGCA-3′</td>
<td>5′-TGTGCAATTTGCAATCCTG-3′</td>
</tr>
<tr>
<td>Stat3</td>
<td>5′-TGTTGCTCCAGCGACATGCA-3′</td>
<td>5′-GTTCAGCTATTTAGCTGCA-3′</td>
</tr>
<tr>
<td>Ccl2</td>
<td>5′-GGAGACAGTGAAAAAGCTTG-3′</td>
<td>5′-CCTGAAAGGATCAGTGATCT-3′</td>
</tr>
<tr>
<td>Cxcl1</td>
<td>5′-TACAAACACAGCCTCCCC-3′</td>
<td>5′-TACAAACACAGCCTCCCC-3′</td>
</tr>
<tr>
<td>Foxp3</td>
<td>5′-GGTCGTTCTCATGACC-3′</td>
<td>5′-TACATGCTGTAACAGAGGC-3′</td>
</tr>
<tr>
<td>Reg3y</td>
<td>5′-TCTGATCCTTCACTACGAACG-3′</td>
<td>5′-GATCATGCTGTAACAGAGGC-3′</td>
</tr>
<tr>
<td>Ahr</td>
<td>5′-ATCACATCTATGTCGGCGGC-3′</td>
<td>5′-GATCATGCTGTAACAGAGGC-3′</td>
</tr>
<tr>
<td>Muc1</td>
<td>5′-ATCCATCATACACCTGCCCTG-3′</td>
<td>5′-ATCCATCATACACCTGCCCTG-3′</td>
</tr>
<tr>
<td>Muc2</td>
<td>5′-GTCGTTCTTCACTACGAACG-3′</td>
<td>5′-ATCCATCATACACCTGCCCTG-3′</td>
</tr>
<tr>
<td>Ahr</td>
<td>5′-ATCACATCTATGTCGGCGGC-3′</td>
<td>5′-GATCATGCTGTAACAGAGGC-3′</td>
</tr>
<tr>
<td>Muc1</td>
<td>5′-ATCCATCATACACCTGCCCTG-3′</td>
<td>5′-ATCCATCATACACCTGCCCTG-3′</td>
</tr>
<tr>
<td>Muc2</td>
<td>5′-GTCGTTCTTCACTACGAACG-3′</td>
<td>5′-ATCCATCATACACCTGCCCTG-3′</td>
</tr>
<tr>
<td>Ahr</td>
<td>5′-ATCACATCTATGTCGGCGGC-3′</td>
<td>5′-GATCATGCTGTAACAGAGGC-3′</td>
</tr>
<tr>
<td>foxp3</td>
<td>5′-GGTCGTTCTCATGACC-3′</td>
<td>5′-TACATGCTGTAACAGAGGC-3′</td>
</tr>
<tr>
<td>reg3y</td>
<td>5′-TCTCAGAACGGGAC-3′</td>
<td>5′-GATCATGCTGTAACAGAGGC-3′</td>
</tr>
<tr>
<td>Ahr</td>
<td>5′-ATCACATCTATGTCGGCGGC-3′</td>
<td>5′-GATCATGCTGTAACAGAGGC-3′</td>
</tr>
</tbody>
</table>

3. Results

3.1. Tryptamine content in feces

HPLC analysis and the extraction procedure were performed as described previously, with slight modifications [28]. Briefly, linear gradients were applied and separations were performed with 10 mM HCOONH4 (pH 3.4) from 0 to 5 min and then a linear gradient of 0%–25% acetonitrile in 10 mM HCOONH4 (pH 3.4) from 5 to 15 min; the run was continued for column washing (90% acetonitrile for 9 min), followed by reequilibration [10 mM HCOONH4 (pH 3.4) for 8 min]. The total HPLC run time for each sample was 32 min, and the injection volume was 20 μl in an Atlantis C18 column (4.6×50 mm, 5 μm; Waters, Milford, MA, USA) at 30 °C at a flow rate of 1.0 ml/min. Fluorescence was detected using 300- and 355-nm excitation and emission, respectively.

3.2. Quantitative RT-PCR

RNA was isolated from colon tissue by using the commercially available reagent IsoGen (Nippon Gene, Co., Ltd., Tokyo, Japan), and then RNA cleanup was performed using the RNeasy mini kit (QIAGEN GmbH, Hilden, Germany) with optional DNasel treatment, according to the manufacturer’s instructions.
After RNA isolation, the ratio of absorbance at 260 and 280 nm was determined, and 4 µg of RNA was used to synthesize cDNA as previously described [14]. Aliquots of the obtained cDNA were then used as templates for the subsequent quantitative PCR performed using a 7300 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA).

The target cDNAs were amplified using gene-specific primers and SYBR Premix Ex Taq (Takara Bio, Otsu, Japan). The relative gene expression levels were normalized to the amount of eukaryotic elongation factor-1α (EEF1A1) mRNA [14]. The primers used in the study are listed in Table 1.

2.8. Statistical analyses

Data are presented as means ± standard error of the mean (S.E.M.). SigmaPlot version 12.5 (San Jose, CA, USA) was used for statistical analysis. Two-way analysis of variance (ANOVA) was used to compare the effects of the diet difference (control and 0.5% tryptophan) on the two genotypes (WT and KO), and when significant interactions were observed, individual means of columns were compared using Tukey’s multiple comparison. Repeated-measure-based parameters (such as body weight change over time) were analyzed using two-way ANOVA for repeated measures followed by Tukey’s test. P < .05 was considered statistically significant.

3. Results

3.1. General observation of colitis

Average food and tryptophan intake and DSS-containing water consumption did not differ in a statistically significant manner among the groups (Supplementary Fig. 1). In mice exposed to DSS, colitis symptoms, for example, body weight lowering and bloody stools, increased markedly after Day 4 and reached maximal severity at Day 8 in all groups except the WT group that received the tryptophan diet. As a result, food and water consumption was affected in all groups except the WT group receiving tryptophan diet after Day 4. The body weight change measured for the different groups is shown in Fig. 1A, and these findings agree with the results of the DAI measurement (Fig. 1B). Watery stool was detected and rectal bleeding was severe in mice that received the control diet and in the KO mice that received the tryptophan diet. At the end of the observation period, all mice were

Fig. 1. General observation of colitis. Body weight change (%) (A). The rate of body weight gain or loss in each mouse was calculated using this formula: Body weight change (%) = [(Weight each day) − (Weight at Day 0)] / (Weight at Day 0) × 100 [26]. Disease activity index (B). Colon length (C), spleen length (D) and spleen weight (E). For panels A and B, two-way repeated ANOVA was performed, and values are expressed as means ± S.E.M. (n = 7–8). For panels C–E, two-way ANOVA was performed, and then individual means were compared within groups by using the Tukey multiple-comparison test; values are expressed as means ± S.E.M. (n = 7–8). Different letters indicate significant differences. g, Genotype; d, diet; 2WA g×d, 2-way ANOVA with a significant interaction between genotype and diet.
sacrificed and all colons were collected for measuring their length, and spleen length and weight in order to determine tryptophan association with colonic inflammation. In all groups except the WT group that received the tryptophan diet, the colon was significantly shortened and spleen length and weight were significantly increased (Fig. 1C–E).

3.2. Tryptophan diet reduces histopathological score and levels of MPO activity and TBARS

Histopathological analyses were performed to investigate morphological changes such as epithelial abrasions, crypt disturbance and inflammatory cell infiltration in mucosal and submucosal areas of the colon. Representative H&E-stained sections are shown in Fig. 2A–D. Epithelial outer-layer disruption, crypt-cell damage and inflammatory cell infiltration into the mucosa and submucosa were observed in the groups that received the control diet and in the KO group that received tryptophan diet (Fig. 2A–B, D), whereas a stable epithelial outer layer was detected in the WT group that received the tryptophan diet (Fig. 2C). The histological score was higher in the groups that received the control diet and in the KO group that received the tryptophan diet than in the WT group that received the tryptophan diet (Fig. 2E).

We measured MPO activity in the colon and found that the levels were increased in mice that received the control diet and even in the KO mice on the tryptophan diet, but not in WT mice on the tryptophan diet (Fig. 2F). Moreover, the levels of TBARS, an oxidative stress marker, were significantly higher in all groups as compared with the level in the WT group that received the tryptophan diet (Fig. 2G).

3.3. Tryptophan diet suppresses proinflammatory cytokines and chemokines in WT mice

All animals treated with DSS showed significantly increased levels of the proinflammatory cytokines Tnfrf and Il-6 in the serum as
compared to the levels in the WT mice that received the tryptophan diet (Fig. 3A, B). To investigate the role of tryptophan supplementation on local immune responses, we used quantitative RT-PCR to measure the mRNA expression of proinflammatory cytokines and inflammatory mediators involved in the pathogenesis of colitis. The mRNA levels of Il-6, Tnfα, Il-1β, Ccl2, Cxcl11 and Cxcr3 were elevated in the groups that received the control diet and in the KO group that received the tryptophan diet, but was decreased in the WT group that received the tryptophan diet (Fig. 3C–H).

3.4. Tryptophan diet increases Ahr ligand levels and Il-22 and Stat3 mRNA expression

Fresh feces were collected for tryptamine determination before DSS administration. Tryptamine levels were increased in both the WT and KO groups that received the tryptophan diet but not the control diet (Fig. 4A). Serum and colonic tryptamine levels were measured at the end of the DSS administration (Fig. 4B, C), and the tryptamine level in the colon was found to be higher in the tryptophan diet groups than in the control diet groups (Fig. 4C). Serum tryptophan level was found higher only in tryptophan-receiving groups (Fig. 4D). We further characterized the effect of Ahr and the tryptophan diet during colitis, and our results showed that the tryptophan diet significantly enhanced the mRNA level of Ahr in WT mice (Fig. 4E), and that the induction of Ahr also led to increased Il-22 and Stat3 expression (Fig. 4F, G). Furthermore, Il-22/Stat3 induction resulted in increased mRNA levels of the antibacterial peptide Reg3γ and mucus-associated mucins in the WT group that received the tryptophan diet, but not in other groups (Fig. 5A–E). We also measured the mRNA levels of Foxp3 and Il-17 to examine the adaptive role of Ahr induction by tryptophan; the tryptophan diet up-regulated Foxp3 expression but downregulated Il-17 mRNA expression only in the WT group (Fig. 5F, G).

4. Discussion

We have demonstrated a mechanism by which L-tryptophan protects against DSS-induced colitis in mice. Tryptophan supplementation reduced body weight loss, disease activity indices and local inflammatory-cytokine expression and improved epithelial structural integrity in WT but not Ahr KO mice. This is because tryptophan metabolites, not tryptophan itself, act as ligands for Ahr [41] and their binding causes Ahr to regulate intestinal immunity [10,11,25]. Generally, only a small fraction of L-tryptophan is converted into tryptamine by intestinal flora, but this amount is substantially...
increased after L-tryptophan administration [16]. Fecal excretion of tryptamine was found to be similar in both tryptophan diet groups before DSS treatment, but colonic tryptamine levels were relatively higher in WT than KO groups, although both groups received tryptophan, which might be due to dysbiosis in the KO group after DSS treatment.

Direct epithelial cell damage by DSS triggers abnormal intrusion of microbial flora into the cell, which is responsible for initiating innate immune responses and leads to the production of abundant reactive oxygen species [18,29]. In experimental colitis, disease severity is frequently correlated with an increase in MPO activity and elevated levels of systemic proinflammatory cytokines [2,26,30]. MPO is commonly used as a surrogate marker of inflammation and is representative of gut permeability. Here, colonic MPO and TBARS were significantly increased in the control diet groups and the tryptophan diet KO group, but not in the WT group that received the tryptophan diet. Moreover, the serum levels of the proinflammatory cytokines Tnfa and Il-6 were decreased only in the WT group that received the tryptophan diet. Chemokines are also responsible for colitis severity as because they regulate the trafficking and migration of leukocytes [2]. Colonic mRNA expression Tnfa and Il-6 and the chemokines Ccl2 and Cxcl1 and the chemokine receptor Cxcr3 were higher in all of the groups as compared with the level in the WT group that received the tryptophan diet. These findings suggest that tryptophan produces an anti-inflammatory effect in DSS-induced colitis through Ahr. Tryptophan diet markedly increased Ahr mRNA expression in WT mice but not in the Ahr KO mice, and the lack of protective effect on the KO mice also revealed that the inability of the endogenous Ahr ligands to act as bioregulatory molecules in the KO groups resulted severe colitis.

In histological analysis, colonic inflammation is characterized by crypt cell damage, mucosal ulceration, erosion and neutrophil infiltration into the mucosal tissue [31]. Similar findings were obtained here in the case of control diet groups and the tryptophan diet KO group, but not the WT group that received the tryptophan diet. Thus, tryptophan supplementation protects the epithelial layer during acute colitis and contributes to the prevention of the intestinal inflammation [32]. In normal healthy gut, Ahr is necessary for the
organogenesis of postnatal lymphoid tissues, the maintenance/ expansion of intraepithelial lymphocytes cells and IL-22-producing ILCs [33]. Thus, Ahr activation up-regulates IL-22 production, which is crucial for epithelial layer integrity. In a model of experimental Citrobacter rodentium infection, IL-22−/− mice showed severe intestinal epithelial layer damage, together with systemic bacterial burden and substantially increased mortality [34]. In this study, we found that colonic IL-22 mRNA expression was increased in the WT group that received the tryptophan diet. At the initial stage of epithelial cell damage, the recruitment of granulocytes and macrophages was correlated with a notable increase in IL-22 expression by ILCs in the middle and distal colon, which is critical for limiting further epithelial cell damage [18]. However, additional investigation is required to elucidate the role of tryptophan in inducing IL-22 production by either directly regulating IL-22 expression or regulating the production and development of type 3 ILCs through Ahr. The KO mice exhibited a considerable deficit in ILCs, which resulted in diminished IL-22 secretion and inadequate protection against intestinal bacterial infection [15]. Activated IL-22 also increased the colonic mRNA expression of Stat3 [35], which is similar to what we found here.

Fig. 5. Effect of tryptophan diet on antibacterial peptide levels and adaptive immunity. Colonic mRNA expression of Reg3γ (A), mucins (B–E), Foxp3 (F) and IL-17 (G) was determined using qRT-PCR. Two-way ANOVA was performed and individual means were compared using the Tukey multiple-comparison test; values are expressed as means±S.E.M. (n=3). Different letters indicate significant differences. g, Genotype; d, diet; 2WA g×d, 2-way ANOVA with a significant interaction between genotype and diet.

Stat3, a key modulator of tissue homeostasis after colitis induction, regulates the expression of the antibacterial peptide Reg3γ and the formation of the mucus layer through mucin gene expression [36,37]. Our study has provided evidence that Ahr-mediated induction of IL-22/Stat3 enhanced the expression of Reg3γ and up-regulated Muc1, Muc2, Muc3 and Muc4 in the WT group that received the tryptophan diet but not in the control diet groups or in the KO mice that received the tryptophan diet. Thus, activated Ahr-mediated IL-22/Stat3 induction plays a crucial role in mucosal epithelial homeostasis and integrity.

Ahr signaling plays an integral role in adaptive immunity [38]. Here, Ahr up-regulated the expression of Foxp3 in the WT group receiving on the tryptophan diet. Foxp3 programs the development and function of the CD4+CD25+ Tregs that are essential for the active suppression of autoimmunity [39]. However, the colonic mRNA expression of IL-17 produced by Th17 cells was higher in all groups produced by Th17 cells was higher in all groups.
effectors and regulatory T cells through Ahr. Distinct ligands and their affinity for Ahr might determine whether Th17 cells or Tregs are induced [40]; further studies are required to uncover the role of Ahr in regulating Tregs and Th17 cells involving the use of tryptophan diet.

In conclusion, our findings delineate a previously unrecognized mechanism by which the production of inflammatory cytokines and pathology can be inhibited in the gut through the activation of Ahr by a 0.5% tryptophan diet and the subsequent induction of the IL-22–Stat3 pathway. These findings not only represent the precise relationship between tryptophan metabolites and Ahr, but also verify that tryptophan supplementation could be considered an optimizing candidate for IBD prevention and treatment.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jnutbio.2016.12.019.

Acknowledgment

This work was partially supported by a grant from the research project on the development of agricultural products and foods with health promoting benefits (National Agriculture and Food Research Organization), Japan. The authors declare that they have no conflict of interests.

References