Biochemical and Biophysical Research Communications 446 (2014) 529–534

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Induction of TGF-β and IL-10 production in dendritic cells using astilbin to inhibit dextran sulfate sodium-induced colitis

Yanbing Ding [a,*,1], Yu Liang [a,1], Bin Deng [a,1], Ahui Qiao [a], Keyan Wu [a], Weiming Xiao [a], Weijuan Gong [b]

[a] Department of Gastroenterology, The Second Clinical College of Yangzhou University, 45 Taizhou Road, Yangzhou, Jiangsu 225001, China
[b] Department of Immunology, School of Medicine, Yangzhou University, 11 Huaihai Road, Yangzhou, Jiangsu 225001, China

ARTICLE INFO

Article history:
Received 19 February 2014
Available online 12 March 2014

Keywords:
Astilbin
Dendritic cells
Colitis

ABSTRACT

Astilbin, a major bioactive compound from *Rhizoma smilacis glabrae*, has been reported to possess anti-inflammatory properties. Our study first evaluated astilbin on dextran sulfate sodium (DSS)-induced acute colitis in mice. By intraperitoneal injection of astilbin, the severity of colitis was attenuated, and the serum levels of IL-10 and TGF-β were increased. Using flow cytometry, a higher number of IL-10$^+$ dendritic cells (DCs) and TGF-β$^+$ DCs and a lower number of CD86$^+$ DCs, IL-12 p40$^+$ DCs, and IL-1β$^+$ DCs were detected in the spleen of mice with colitis after astilbin treatment. The administration of astilbin also resulted in the upregulation of CD103$^+$ expression in colonic DCs. In a coculture system, murine bone marrow-derived DCs pretreated with astilbin resulted in an enhanced production of CD4$^+$CD25$^+$Foxp3$^+$ T cells. The results of this study show that astilbin could be a candidate drug for inflammatory bowel disease by mediating the regulatory functions of DCs.

1. Introduction

Inflammatory bowel disease (IBD), a chronic relapsing nonspecific inflammation in the intestines that involves the overproduction of inflammatory cytokines and a reduction of regulatory cytokines, is difficult to treat. IBD is currently regarded as a global disease [1,2]. Although the precise etiology of IBD is unclear, the pathogenesis of human IBD has been associated with an inappropriate and exaggerated immune response to gut flora in a genetically predisposed individual [3,4]. Despite the increase in the number of therapeutic options for disease management, IBD therapies have significant limitations with regard to safety, efficacy, and applicability [5–7].

The most useful strategy for IBD treatment is the blockade of the abnormal immune responses of patients. However, the use of immunosuppressive reagents is limited by their lack of antigen specificity, which results in a general suppression of immune function and the occurrence of various side effects such as infection and cancer susceptibility [8–10]. To minimize these side effects, several studies have focused on the suppression of antigen-specific immune responses, especially involving the maturation and function of dendritic cells (DCs). DCs are highly efficient antigen-presenting cells that capture, process, and present antigens to T cells [11]. In certain conditions (e.g., cancer and chronic infections), DCs induce anergy in antigen-specific T cells and promote the generation of Foxp3$^+$ regulatory T cells (Treg) [12–15].

A number of plant-derived natural products have the ability to suppress deleterious immune responses in patients with fewer side effects [16]. These reagents are also called biological response modifiers (BRMs) [17,18]. Astilbin, a flavanone isolated from *Rhizoma smilacis glabrae*, imparts biological activities such as antioxidant [19], insecticidal [20], antimicrobial [21,22], antinociceptive [23], antiedematogenic [23], anti-inflammatory [24], and immunosuppressive effects. The immunosuppressive property of astilbin involves the suppression of lymphocyte function [25], inhibition of T-lymphocyte adhesion [26], and stimulation of IL-10 production [27]. In the present study, we tested whether the immunosuppressive activity of astilbin was associated with the regulation of DC function using a dextran sodium sulfate (DSS)-induced murine colitis model. We examined the immunomodulatory effects of astilbin on DCs both *in vivo* and *in vitro* and provide additional evidence that may help in the elucidation of the molecular mechanism of astilbin for the treatment of IBD.

2. Materials and methods

2.1. Animals and cell lines

C57BL/6 mice aged of 6–8 weeks were purchased from the Comparative Medicine Centre of Yangzhou University (Yangzhou,

* Corresponding author.
E-mail address: yanbingding66@126.com (Y. Ding).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.02.136
0006-291X/

China). The care and use of the animals and the experiment protocol were approved by the Animal Experiment Ethics Committee of Yangzhou University (Permission number: 20120405). The RAW264.7 cell line was purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). The cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 units/mL of penicillin, and 100 μg/mL of streptomycin sulfate, and maintained in a standard incubator with humidified air containing 5% CO_2 at 37 °C.

2.2. Drugs and reagents

Astilbin (3,3′,4′,5,7-pentahydroxyflavanone 3-(6-deoxy-(L-mannopyranoside)), purity >98%) was purchased from Tianjin Phytomarker Ltd (Tianjin, China). RPMI 1640 medium, DMEM medium, and FBS were obtained from Thermo (USA). Penicillin-streptomycin Solution and red blood cell lysis buffer were purchased from Beyotime (Jiangsu, China). Dextran sodium sulfate (DSS) (molecular weight, 36,000–50,000) was obtained from MP Biomedical (Solon, Ohio, USA). Lymphoprep was obtained from Axis-shield (Oslo, Norway). Collagenase IV, Dnase I, DL-dithiothreitol, and Percoll were purchased from BIOSHARP (USA). Dispase II was obtained from Roche (Basel, Switzerland).

2.3. Antibodies and flow cytometry analysis

The antibodies for flow cytometry were obtained from Biolegend (San Diego, CA, USA) or eBioscience (San Diego, CA, USA). We used antibodies against human CD86 (IT2.2), CD11c (3.9), IL-12 p40 (C11.5), IL-1β (CRM56), IL-10 (JES3-19F1), TGF-β (TW4-2F8) and antibodies against mouse CD86 (GL-1), CD11c (N418), TGF-β (TW7-16B4), IL-1β (NJTEN3), IL-12 p40 (C17.8), IL-10 (JES5-16E3), CD103 (2E7), CD4 (RM4-5), CD25 (PC61.5), and Foxp3 (NRRF-30). Mouse IgG2b, κ Isotype Ctrl (MPC-11), Rat IgG2a, κ Isotype Ctrl (RTK2758), Mouse IgG1, κ Isotype Ctrl (MOPC-21), Rat IgG1, κ Isotype Ctrl (RTK2071), Rat IgG2b, κ Isotype Ctrl (RTK4530). Neutralized TGF-β antibody (1D11), mouse IL-10, TGF-β, and IL-1β ELISA kits were obtained from R&D Systems (Minneapolis, MN). Anti-mouse CD4 and CD11c MicroBeads were purchased from Miltenyi Biotec (Krohne, German). Brefeldin A (BFA) and FIX&PERM KIT were obtained from eBioscience. Cell Apoptosis 7-amino-actinomycin D (7-AAD) Detection Kit was purchased from KeyGEN (Jiangsu, China). Recombinant human and murine GM-CSF and IL-4 were purchased from Peprotech (Rocky Hill, NJ, USA).

After a 30-min incubation with the Fc Block, the cells were incubated with the appropriate antibody at 4 °C for 30 min. Detection of intracellular cytokine need to be fixed and permeabilized in advance by the FIX&PERM kit. The cells were then washed and analyzed by flow cytometry using Cellquest (BD Calibur).

2.4. Induction and evaluation of acute colitis in mice

To induce acute colitis, DSS (2.5%) was added to drinking water from day 1 to day 7. Astilbin (25, 50 mg/kg) or a vehicle was administered daily through intraperitoneal injection. On day 8, the mice were sacrificed. Segments of the colon were fixed in formalin, embedded in paraffin, sectioned at 4-μm thickness, and stained with hematoxylin and eosin (H&E). To assess the extent of colitis, loss in body weight (0, none; 1, 1% to 5%; 2, 5% to 10%; 3, 10% to 20%; and 4, >20%), stool consistency (0, normal; 2, loose stools; and 4, watery diarrhea), and blood in the stool (0, normal; 2, slight bleeding; and 4, gross bleeding) were monitored daily by trained individuals blinded to the treatment groups. Disease activity scores are calculated using the total score, which ranged from 0 to 12.

2.5. Measurement of levels of serum cytokines by ELISA

Mice were anesthetized and peripheral blood was collected from the orbit. Serum was separated by centrifugation and used for the measurement of IL-10, TGF-β, and IL-1β by ELISA according to the manufacturer's protocol.

2.6. Isolation of murine lamina propria mononuclear cells (LPMCs) from the colon

The colon of dead mice was removed and any feces were removed by flushing with PBS using a syringe. The tissue was opened longitudinally, cut into 5-mm pieces, and incubated in 5-mM EDTA and 1-mM DTT in calcium-free and magnesium-free Hank's balanced salt solution for 20 min at 37 °C. The tissue was then centrifuged and the supernatant was collected. The residual tissue was incubated again for 30 min at 37 °C in a digestion solution containing 0.5 mg/mL of each of type IV collagenase and Dnase I, as well as 3 mg/mL of Dispase II. The suspension was resuspended and combined with the previous supernatant with 40% Percoll and placed in 80% Percoll solution. LPMCs were obtained at the 40–80% interface through centrifugation. 7-AAD staining was performed to exclude dead cells.

2.7. Magnetic cell sorting (MACS)

Splenic $CD11c^+$ cells and $CD4^+$ T cells were obtained by MACS. Splenocytes were suspended in 100 μL of PBE (PBS + 0.5% BSA + 2-mM EDTA) containing 20 μL of microbead antibody and incubated at 4 °C for 20 min. After washing, the cells were applied onto a MACS MS column and placed on the MACS separator. The column was then removed from the separator, 1 mL of PBS was added, and the cells were eluted out. The eluent was used in assays when the purity was >95%.

2.8. In vitro stimulation of human monocyte-derived DCs

The human subject research was approved by the human subject experiment Ethics Committee of Yangzhou University (Permission number: 20120307). The blood samples of patients were obtained after obtaining a written informed consent. Peripheral blood mononuclear cells (PBMCs) were isolated from the buffy coat of blood samples donated by IBD patients according to the instructions. The PBMCs were cultured with complete RPMI 1640 medium containing 100 ng/mL of rhGM-CSF and 40 ng/mL of rhIL-4. On day 5, the immature DCs were collected and seeded onto a 24-well plate at a density of 2×10^5/mL. These cells were further incubated with 100 ng/mL rhGM-CSF, 40 ng/mL rhIL-4, and different levels of astilbin for 24 h.

2.9. Incubation $CD4^+$ T cells with murine bone marrow-derived DCs (BM-DCs)

The femurs and shins of the sacrificed mice were collected and BM was flushed out with medium. The collected marrow was resuspended in an erythrocyte lysis solution and centrifuged. After centrifugation, the cells were incubated in a complete medium containing 10 ng/mL of rmGM-CSF and 4 ng/mL of rmIL-4. On day 3, the nonadherent cells were removed and a complete medium with 10 ng/mL of rmGM-CSF and 4 ng/mL of rmIL-4 was added. BM-DCs were harvested on day 5 and analyzed for CD11c expression. BM-DCs were used in assays when the frequency of $CD11c^+$ was >90%. BM-DCs were treated with different levels of astilbin for 24 h and then washed with RPMI 1640 medium. BM-DCs pretreated by astilbin were combined with splenic $CD4^+$ T cells isolated by MACS and supplemented with 10 ng/mL of rmIL-2.

Cells were sufficiently mixed and incubated at 37 °C for 48 h. Finally, the number of $CD4^+CD25^+Foxp3^+$ T cells was determined by flow cytometry.

2.10. Statistical analysis

Results were expressed as means ± SEM of n experiments. Students' *t*-test was used to compare a single treatment mean with a control mean. Data were evaluated by one-way analysis of variance followed by Dunnett's test between control group and multiple dose groups. A *P* value of <0.05 was considered significant.

3. Results

3.1. Astilbin suppressed the severity of DSS-induced colitis in mice

The effect of astilbin on the development of murine colitis induced by DSS was examined first. The body weight and disease activity score were used to evaluate illness severity. As shown in Fig. 1A, the astilbin group showed a smaller decrease in body weight. Disease activity score that reflects the severity of weight loss, intestine bleeding, and stool consistency were significantly lower in the astilbin group. The macroscopic changes of inflamed colons with different treatments were shown in Fig. 1B. Histopathological changes in the colons in all groups were also evaluated. Compared with the vehicle, the colonic sections of DSS-treated mice showed cryptic distortion, massive inflammatory infiltration, and loss of mucosal membrane cells, leukocyte infiltration, and lack of integration in the glandular areas, whereas astilbin remarkably attenuated the extent and severity of the histological signs of colitis (Fig. 1C).

3.2. Induced generation of regulatory DCs and $CD4^+CD25^+Foxp3^+$ T cells in mice with DSS-induced colitis by astilbin treatment

We examined the serum levels of IL-10, TGF-β, and IL-1β in DSS-treated mice. Astilbin treatment resulted in an increase in the serum level of IL-10 and TGF-β and a decrease in the serum level of IL-1β (Fig. 2A). Compared with the DSS group, mean fluorescence intensity (MFI) of CD86, IL-1β, and IL-12 p40 of the splenic DCs in mice with colitis and treated with astilbin was decreased. Furthermore, MFI of IL-10 and TGF-β of splenic DCs was increased by astilbin treatment (Fig. 2B and C). The figures of isotype control were shown in supplementary material. The frequency of splenic $CD4^+CD25^+Foxp3^+$ T cells was also increased in astilbin 50 mg/kg group (Fig. 2D). $CD103^+$ DCs in the intestinal mucosa play a crucial role in the development of tolerance to commensal bacteria and food antigens [28,29]. We observed that astilbin promotes $CD103^+$ expression in colonic DCs from mice with DSS-induced colitis (Fig. 2E and F).

3.3. Astilbin induced DCs and RAW264.7 cells to secrete IL-10 and TGF-β ex vivo

To confirm the effect of astilbin on the induction of regulatory DCs, splenic $CD11c^+$ cells of healthy mice sorted by MACS were cul-

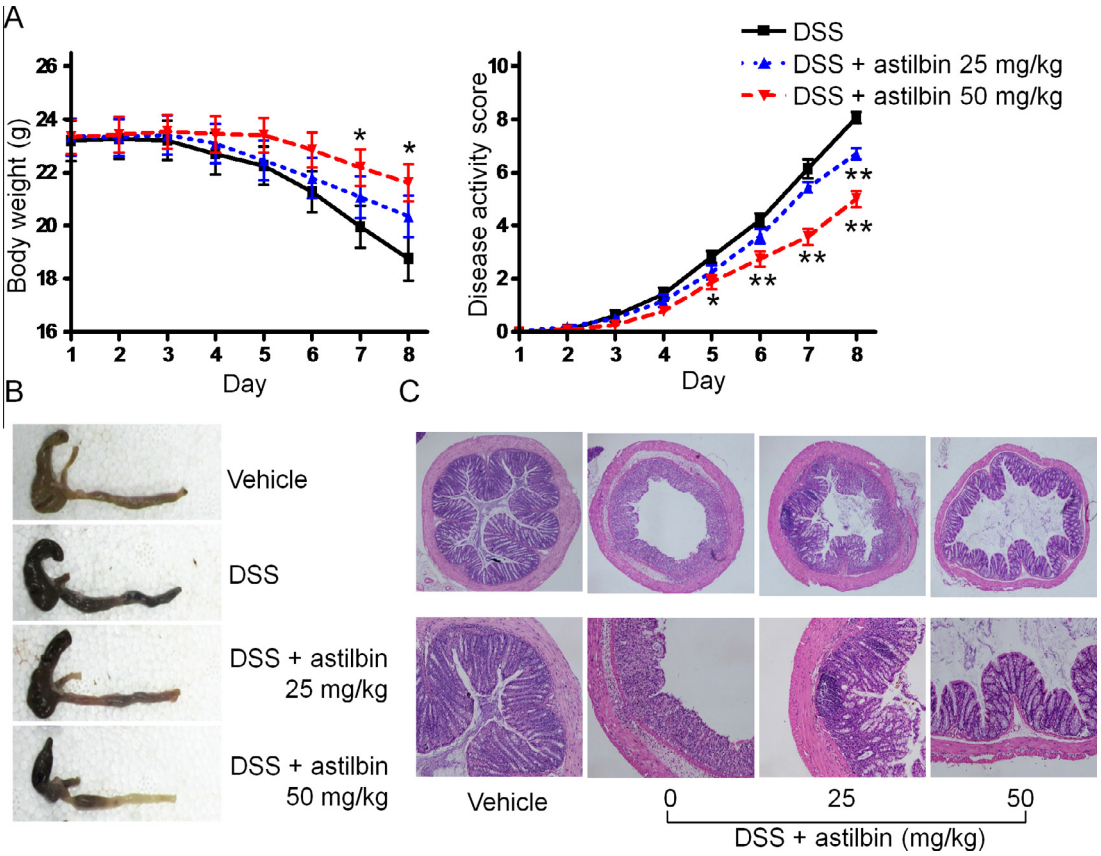

Fig. 1. Astilbin ameliorated the severity of DSS-induced colitis in a mouse model. Murine colitis was induced by orally administering 2.5% DSS from day 1 to day 7. Astilbin (25 or 50 mg/kg) or vehicle was administered through intraperitoneal injection from day 1 to day 7. Mice were sacrificed on day 8. (A) The body weight and the disease activity level of each group was recorded. (B) A representative view of the macroscopic colon among the different treatments. (C) Histopathological changes of inflamed colons using different treatments. Original magnification 40× (upper panel) and 100× (lower panel). All data are presented as mean ± SEM for each group. Each experiment was performed four times (*$P < 0.05$; **$P < 0.01$).

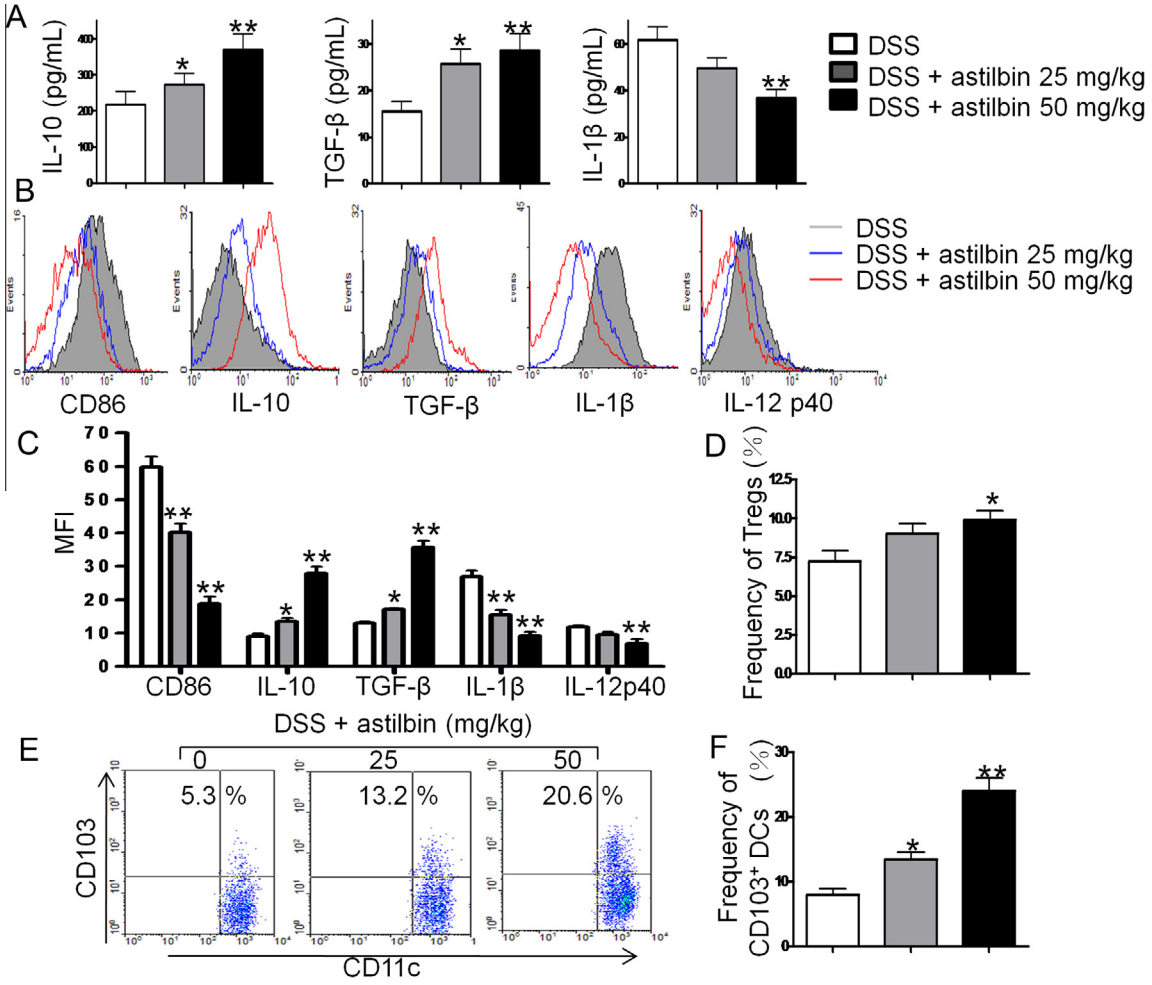

Fig. 2. Induced regulatory DCs and $CD4^+CD25^+Foxp3^+$ T cells in mice with DSS-induced colitis after astilbin treatment. (A) The levels of serum cytokines IL-10, TGF-β, and IL-1β in DSS-treated mice were detected by ELISA. (B) The surface expression of CD86 in splenic DCs was detected by flow cytometry gated on $CD11c^+$ cells. IL-10, TGF-β, IL-1β and IL-12 p40 secretion in splenic DCs was detected by intracellular staining methods gated on $CD11c^+$ cells. (C) Statistical graph of mean fluorescence intensity (MFI). (D) Statistical analysis of the frequency of splenic $CD4^+CD25^+Foxp3^+$ T cells. (E) The surface expression of CD103 of colonic DCs was detected by flow cytometry gated on $CD11c^+$ cells. (F) Statistical graph of frequency of $CD103^+$ DC. All data are presented as mean ± SEM for each group. Each experiment was repeated thrice ($^*P < 0.05$; $^{**}P < 0.01$).

tured with different levels of astilbin. Variations in CD86 expression and cytokine secretion were measured. CD86 was downregulated in a dose-dependent manner, whereas IL-1β and IL-12 p40 secretion in DCs was inhibited by astilbin treatment. Furthermore, the production of IL-10 and TGF-β in DCs was significantly increased after astilbin treatment (Fig. 3A). Considering the purity (95%) of the sorted $CD11c^+$ cells of the spleen, we stimulated a macrophage cell line, RAW264.7, with different levels of astilbin to rule out the effects of other cells in our culture system. As expected, the RAW264.7 cells showed a decreased secretion of IL-1β and IL-12 p40 and an increased secretion of IL-10 and TGF-β after astilbin stimulation (Fig. 3B). Human monocyte-derived DCs from IBD patients were also detected. Astilbin could promote IL-10 and TGF-β production in patients' monocyte-derived DCs. In addition, astilbin downregulated CD86 expression and promoted IL-1β and IL-12 p40 production (Fig. 3C). The effect of the highest dose of astilbin on DCs is shown in Fig. 3. The results of the other doses and the isotype controls are presented as the Supplementary material.

3.4. DCs pretreated with astilbin induced the generation of Treg cells

Because TGF-β is a key cytokine for Treg cell development *ex vivo*, we next examined whether BM-derived DCs (BM-DCs) pretreated with astilbin could promote induction of regulatory $CD4^+CD25^+Foxp3^+$ T cells. After astilbin treatment, BM-DCs were cocultured with splenic $CD4^+$T cells for 48 h, and absolute cell numbers of $CD4^+CD25^+Foxp3^+$ T cells were then calculated. $CD4^+CD25^+Foxp3^+$ T-cell numbers were significantly enhanced in a dose-dependent manner (Fig. 4A and B). When a neutralized TGF-β monoclonal antibody was added to the cell coculture system, the induction of $CD4^+CD25^+Foxp3^+$ T cells was inhibited. Thus, the induction of regulatory T cells by DCs pretreated with astilbin was partly through the secretion of TGF-β.

4. Discussion

In this study, we provided the experimental evidence that astilbin could induce IL-10 and TGF-β production in DCs to suppress the severity of DSS-induced colitis in mice. Astilbin treatment resulted in a higher number of $IL\text{-}10^+$ DCs and $TGF\text{-}\beta^+$ DCs and a lower number of $CD86^+$ DCs, $IL\text{-}1\beta^+$ DCs and $IL\text{-}12\ p40^+$ DCs in the spleen of mice with colitis. Astilbin also directly stimulated DCs to secrete IL-10 and TGF-β *ex vivo*, furthermore it inhibited DCs from secreting IL-1β and IL-12 p40. The production of endogenous TGF-β in DCs through astilbin treatment promoted the induction of $CD4^+CD25^+Foxp3^+$ Treg cells; this effect was antagonized by the addition of a TGF-β antibody. Thus, astilbin could serve as a candidate

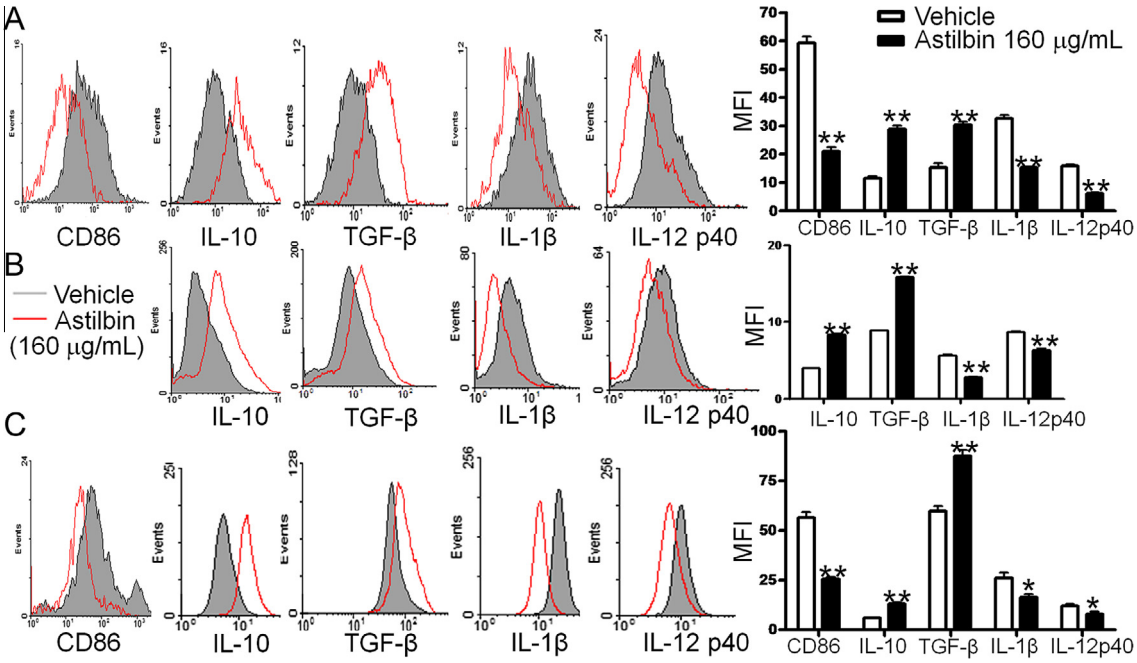

Fig. 3. Astilbin induced DCs and RAW264.7 to secrete IL-10 and TGF-β. (A) Splenic CD11c$^+$ cells of healthy mice sorted by MACS were treated with astilbin for 24 h. The expression of CD86, IL-10, TGF-β, IL-1β, and IL-12 p40 in DCs was measured by flow cytometry gated on CD11c$^+$ cells. (B) RAW264.7 cells were treated with astilbin for 24 h. Production of IL-10, TGF-β, IL-1β, and IL-12 p40 in RAW264.7 cells was detected by intracellular staining. (C) Human monocyte-derived DCs from IBD patients were stimulated with astilbin for 24 h. MFI of CD86, IL-10, TGF-β, IL-1β, and IL-12 p40 in DCs was analyzed by flow cytometry gated on CD11c$^+$ cells. Data shown here are representative of four independent experiments. Each treatment was performed in triplicate ($^*P < 0.05$, $^{**}P < 0.01$).

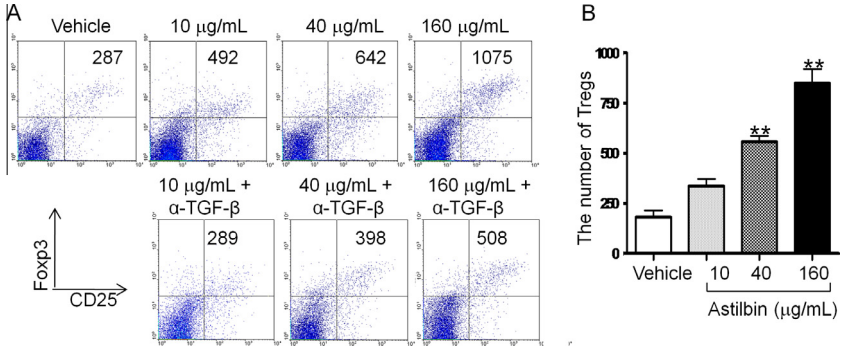

Fig. 4. DCs pretreated with astilbin induced the generation of regulatory T cells. Bone marrow-derived DCs from normal mice were treated with various levels of astilbin for 24 h. Then DCs were cocultured with splenic CD4$^+$ T cells through magnetic sorting for 48 h with or without anti-TGF-β antibody. (A) Regulatory T cells (CD4$^+$CD25$^+$Foxp3$^+$) were detected by flow cytometry. (B) Statistical analysis of the absolute number of CD4$^+$CD25$^+$Foxp3$^+$ T cells is shown. Data are representative of four independent experiments. Each treatment was performed in triplicate ($^*P < 0.05$, $^{**}P < 0.01$).

drug to antagonize local inflammation by inducing DCs to mediate its regulatory functions.

Astilbin directly downregulates the activities of T cells in hypersensitivity [27,30], collagen-induced arthritis [25], and acute heart allograft rejection [31]. Astilbin not only inhibited the proliferation and activation of T cells but also suppressed the adhesion of ConA or PMA-activated Jurkat cells to fibronectin and type IV collagen. In addition, astilbin inhibited the CD44 expression and TNF-α production in Jurkat cells [26]. The molecular mechanism of T-cell suppression by astilbin involves the inhibition of phosphorylation of nuclear factor (NF-κB) and p38 [31]. Although the conditions of T cells in mice with colitis were not analyzed in detail before and after the astilbin treatment, the suppressive effects of astilbin on the murine T cells could not be excluded.

The mature and antigen-presenting functions of DCs were inhibited by astilbin, as determined by morphological observations, flow cytometry, and mixed lymphocyte reaction. Downregulation of CD86 expression and IL-12 p40 production by astilbin in mouse BM-DCs was previously confirmed by Song et al. [32]. Astilbin significantly induced the *in vivo* IL-10 expression of lymph node cells at an earlier time and decreased TNF-α and IFN-γ expression at a later time in contact dermatitis [27]. However, we could not determine which cell produced IL-10, because there are a few DCs in the lymph nodes.

As many suppressive drugs downregulate T-cell functions without antigen specificity, the induction or expansion of antigen-specific Foxp3$^+$ Tregs from the polyclonal T-cell repertoire is an important issue for future clinical therapy [33,34]. Astilbin may act as an efficient therapeutic agent for inflammatory disease such as CsA, but with less toxicity [25,30]. Astilbin mediates its immune suppressive effects not only through the inhibition of T lymphocytes but also through the induction of DCs to mediate regulatory functions. The induction of CD4$^+$CD25$^+$Foxp3$^+$ Treg

cells by DCs pretreated with astilbin suggests that astilbin is safer than conventional approaches.

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder involving the gastrointestinal tract. Current therapeutic options and approaches for IBD continue to evolve. As suggested by mouse models of colitis and by observations in humans, modulation of DC functions has been regarded as an essential player in fine tuning the immune system [35,36]. However, mechanisms involved in the induction of regulatory DCs by astilbin still require further investigations. For example, the actual molecular events occurring in DCs that lead to the induction of IL-10 and TGF-β production after astilbin treatment requires further study.

In conclusion, our study explored the therapeutic effect of astilbin on DSS-induced colitis *in vivo* and *in vitro* through the modulation of DC functions. The effects mediated by astilbin suggest this natural agent can be used in IBD and in other inflammatory diseases such as multiple sclerosis, psoriasis, and rheumatoid arthritis.

Acknowledgments

This work was supported by the National Natural Science Foundation (Nos. 81172785, 30671917), the collaborative fund of Yangzhou University and Yangzhou municipality (2012038-5), the fund of Yangzhou municipality (YZ2011088, YZ2010088), the fund of Jiangsu Province (RC2011044), Program for Postgraduates Research Innovation in University of Jiangsu Province (CXLX13_926).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.02.136.

References

[1] J. Cosnes, C. Gower-Rousseau, P. Seksik, A. Cortot, Epidemiology and natural history of inflammatory bowel diseases, Gastroenterology 140 (2011) 1785–1794.
[2] N.A. Molodecky, I.S. Soon, D.M. Rabi, W.A. Ghali, M. Ferris, et al., Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review, Gastroenterology 142 (2012) 46–54.
[3] B. Khor, A. Gardet, R.J. Xavier, Genetics and pathogenesis of inflammatory bowel disease, Nature 474 (2011) 307–317.
[4] R.J. Xavier, D.K. Podolsky, Unravelling the pathogenesis of inflammatory bowel disease, Nature 448 (2007) 427–434.
[5] D.C. Baumgart, W.J. Sandborn, Inflammatory bowel disease: clinical aspects and established and evolving therapies, Lancet 369 (2007) 1641–1657.
[6] C.A. Siegel, C. Hur, J.R. Korzenik, G.S. Gazelle, B.E. Sands, Risks and benefits of infliximab for the treatment of Crohn's disease, Clin. Gastroenterol. Hepatol. 4 (2006) 1017–1024.
[7] W. Blonski, G.R. Lichtenstein, Safety of biologic therapy, Inflamm. Bowel Dis. 13 (2007) 769–796.
[8] J.R. Kelsen, J. Kim, D. Latta, S. Smathers, K.L. McGowan, et al., Recurrence rate of clostridium difficile infection in hospitalized pediatric patients with inflammatory bowel disease, Inflamm. Bowel Dis. 17 (2011) 50–55.
[9] L. Beaugerie, N. Brousse, A.M. Bouvier, J.F. Colombel, M. Lémann, et al., Lymphoproliferative disorders in patients receiving thiopurines for inflammatory bowel disease: a prospective observational cohort study, Lancet 374 (2009) 1617–1625.
[10] A. Kandiel, A.G. Fraser, B.I. Korelitz, C. Brensinger, J.D. Lewis, Increased risk of lymphoma among inflammatory bowel disease patients treated with azathioprine and 6-mercaptopurine, Gut 54 (2005) 1121–1125.
[11] J. Banchereau, R.M. Steinman, Dendritic cells and the control of immunity, Nature 392 (1998) 245–252.
[12] H. Torres-Aguilar, S.R. Aguilar-Ruiz, G. González-Pérez, R. Munguía, S. Bajaña, et al., Tolerogenic dendritic cells generated with different immunosuppressive cytokines induce antigen-specific anergy and regulatory properties in memory $CD4^+$ T cells, J. Immunol. 184 (2010) 1765–1775.
[13] R. Kushwah, J. Wu, J.R. Oliver, G. Jiang, J. Zhang, et al., Uptake of apoptotic DC converts immature DC into tolerogenic DC that induce differentiation of Foxp3+ Treg, Eur. J. Immunol. 40 (2010) 1022–1035.
[14] S. Khan, J.D. Greenberg, N. Bhardwaj, Dendritic cells as targets for therapy in rheumatoid arthritis, Nat. Rev. Rheumatol. 5 (2009) 566–571.
[15] O. Jaen, S. Rullé, N. Bessis, A. Zago, M.C. Boissier, et al., Dendritic cells modulated by innate immunity improve collagen-induced arthritis and induce regulatory T cells in vivo, Immunology 126 (2009) 35–44.
[16] Q. Ouyang, L.Y. Xue, Inflammatory bowel disease in the 21(st) century in China: turning challenges into opportunities, J. Dig. Dis. 13 (2012) 195–199.
[17] S. Baltatzis, F. Tufail, E.N. Yu, C.M. Vredeveld, C.S. Foster, Mycophenolate mofetil as an immunomodulatory agent in the treatment of chronic ocular inflammatory disorders, Ophthalmology 110 (2003) 1061–1065.
[18] D. Higashi, K. Seki, Y. Ishibashi, Y. Egawa, M. Koga, et al., The effect of lentinan combination therapy for unresectable advanced gastric cancer, Anticancer Res. 32 (2012) 2365–2368.
[19] H. Haraguchi, Y. Mochida, S. Sakai, H. Masuda, Y. Tamura, et al., Protection against oxidative damage by dihydroflavonols in *Engelhardtia chrysolepis*, Biosci. Biotechnol. Biochem. 60 (1996) 945–948.
[20] L.G. Batista Pereira, F. Petacci, J.B. Fernandes, A.G. Correa, P.C. Vieira, et al., Biological activity of astilbin from *Dimorphandra mollis* against *Anticarsia gemmatalis* and *Spodoptera frugiperda*, Pest Manag. Sci. 58 (2002) 503–507.
[21] B. Moulari, Y. Pellequer, J.P. Chaumont, Y.C. Guillaume, J. Millet, In vitro antimicrobial activity of the leaf extract of *Harungana madagascariensis* Lam. Ex Poir. (Hypericaceae) against strains causing otitis externa in dogs and cats, Acta Vet. Hung. 55 (2007) 97–105.
[22] B. Moulari, Y. Pellequer, H. Lboutounne, C. Girard, J.P. Chaumont, et al., Isolation and in vitro antibacterial activity of astilbin, the bioactive flavanone from the leaves of *Harungana madagascariensis* Lam. ex Poir. (Hypericaceae), J. Ethnopharmacol. 106 (2006) 272–278.
[23] V. Cechinel-Filho, Z.R. Vaz, L. Zunino, J.B. Calixto, R.A. Yunes, Antinociceptive and anti-oedematogenic properties of astilbin, taxifolin and some related compounds, Arzneimittelforschung 50 (2000) 281–285.
[24] H. Huang, Z. Cheng, H. Shi, W. Xin, T.T. Wang, L.L. Yu, Isolation and characterization of two flavonoids, engeletin and astilbin, from the leaves of *Engelhardia roxburghiana* and their potential anti-inflammatory properties, J. Agric. Food Chem. 59 (2011) 4562–4569.
[25] Y. Cai, T. Chen, Q. Xu, Astilbin suppresses collagen-induced arthritis via the dysfunction of lymphocytes, Inflamm. Res. 52 (2003) 334–340.
[26] H.W. Yi, X.M. Lu, F. Fang, J. Wang, Q. Xu, Astilbin inhibits the adhesion of T lymphocytes via decreasing TNF-alpha and its associated MMP-9 activity and CD44 expression, Int. Immunopharmacol. 8 (2008) 1467–1474.
[27] M. Fei, X. Wu, Q. Xu, Astilbin inhibits contact hypersensitivity through negative cytokine regulation distinct from cyclosporin A, J. Allergy Clin. Immunol. 116 (2005) 1350–1356.
[28] C.L. Scott, A.M. Aumeunier, A.M. Mowat, Intestinal $CD103^+$ dendritic cells: master regulators of tolerance, Trends Immunol. 32 (2011) 412–421.
[29] R. Zeng, C. Oderup, R. Yuan, et al., Retinoic acid regulates the development of a gut-homing precursor for intestinal dendritic cells, Mucosal Immunol. 12 (2012) 58–67.
[30] Y. Cai, T. Chen, Q. Xu, Astilbin suppresses delayed-type hypersensitivity by inhibiting lymphocyte migration, J. Pharm. Pharmacol. 55 (2003) 691–695.
[31] S. Zou, X. Shen, Y. Tang, Z. Fu, Q. Zheng, Q. Wang, Astilbin suppresses acute heart allograft rejection by inhibiting maturation and function of dendritic cells in mice, Transplant Proc. 42 (2010) 3798–3802.
[32] S.H. Song, X.Y. Shen, G.S. Ding, F. Liu, Z.M. Wang, Z.R. Fu, Effects of astilbin on maturation and immunologic function of mouse bone marrow-derived dendritic cells, Zhong Xi Yi Jie He Xue Bao 8 (2010) 145–151.
[33] A. Izcue, J.L. Coombes, F. Powrie, Regulatory T cells suppress systemic and mucosal immune activation to control intestinal inflammation, Immunol. Rev. 212 (2006) 256–271.
[34] C. Keijzer, R. van der Zee, W. van Eden, F. Broere, Treg inducing adjuvants for therapeutic vaccination against chronic inflammatory diseases, Front. Immunol. 4 (2013) 245.
[35] F. Leon, L.E. Smythies, P.D. Smith, B.L. Kelsall, Involvement of dendritic cells in the pathogenesis of inflammatory bowel disease, Adv. Exp. Med. Biol. 579 (2006) 117–132.
[36] S. Rutella, F. Locatelli, Intestinal dendritic cells in the pathogenesis of inflammatory bowel disease, World J. Gastroenterol. 17 (2011) 3761–3775.