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Decursinol Angelate Ameliorates Dextran Sodium Sulfate-Induced Colitis by Modulating Type 17 Helper T Cell Responses

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Abstract

Angelica gigas has been used as a Korean traditional medicine for pain relief and gynecological health. Although the extracts are reported to have an anti-inflammatory property, the bioactive compounds of the herbal plant and the effect on T cell responses are unclear. In this study, we identified decursinol angelate (DA) as an immunomodulatory ingredient of *A. gigas* and demonstrated its suppressive effect on type 17 helper T (Th17) cell responses. Helper T cell culture experiments revealed that DA impeded the differentiation of Th17 cells and IL-17 production without affecting the survival and proliferation of CD4 T cells. By using a dextran sodium sulfate (DSS)-induced colitis model, we determined the therapeutic potential of DA for the treatment of ulcerative colitis. DA treatment attenuated the severity of colitis including a reduction in weight loss, colon shortening, and protection from colonic tissue damage induced by DSS administration. Intriguingly, Th17 cells concurrently with neutrophils in the colitis tissues were significantly decreased by the DA treatment. Overall, our experimental evidence reveals for the first time that DA is an anti-inflammatory compound to modulate inflammatory T cells, and suggests DA as a potential therapeutic agent to manage inflammatory conditions associated with Th17 cell responses.

Key Words: *Angelica gigas*, Decursinol angelate, Type 17 helper T cell, IL-17, Ulcerative colitis

INTRODUCTION

Alterations in the mucosal microenvironment may disrupt the balance between tolerance and inflammation in the intestinal tract, leading to diseases including inflammatory bowel disease (IBD). IBD is a chronic inflammatory condition of the digestive tract and characterized by symptoms involving diarrhea, abdominal pain, rectal bleeding, and weight loss (Baumgart and Carding, 2007). Ulcerative colitis (UC) and Crohn's disease (CD) are the two of the main types of IBD, and the prevalence of both the diseases has been increasing in the developing world (Neurath, 2014). Moreover, patients with IBD have an increased risk of developing colorectal cancer (Kim and Chang, 2014). Progression of UC and CD involves the erosion of protective epithelial layers causing the exposure of gastrointestinal microbes, dietary antigens and toxins which leads to the activation of immune cells (Gitter *et al*., 2001). Although the mechanisms underlying the development and progression remain unclear, the disruption of immune homeostasis is believed to have a key role in the pathogenesis of IBD.

Proinflammatory cytokines are associated with the etiology of IBD (Strober and Fuss, 2011). Recent studies established that IL-17-producing Th17 cells have a pivotal role in the mucosal immunity in the intestinal tissue (Tesmer *et al*., 2008). It has been reported that proportions of Th17 cells within the intestinal tissue and peripheral blood are increased in the IBD patients (Monteleone *et al*., 2012). Moreover, genetic variations in *IL23R*, *JAK2* and *STAT3*, which promote Th17 cells, are associated with an increased susceptibility to IBD (Neurath, 2014). Several classes of anti-inflammatory agents are currently used in the management of IBD, which include 5-aminosalicylates, azathioprine, corticosteroids and infliximab. However, these drugs have a number of contradictions and side effects, and some of the patients are reported to be resistant to those treatments (de Mattos *et al*., 2015). Thus, targeting Th17 cells in an attempt to control intestinal inflammation may have a potential benefit to treat IBD while minimiz-

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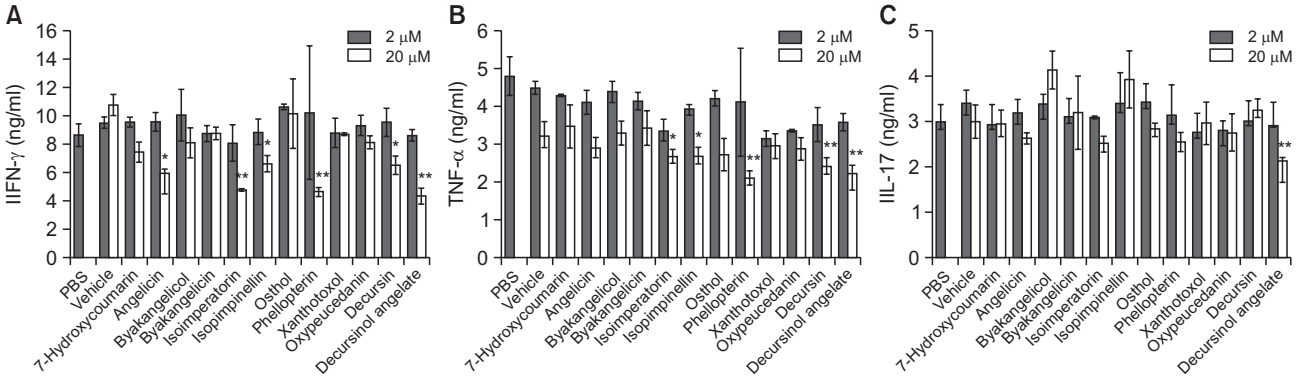

Fig. 1. Decursinol angelate is an immunomodulatory ingredient of *A. gigas*. (A-C) Lymph node cells isolated from OT-II mice were treated with the chemical components of *A. gigas* and activated with ova peptide and culture for 5 days in the presence of IL-2. The culture supernatant was analyzed by CBA. Data represent the mean (± SD) cytokine levels obtained from three independent experiments: **p*<0.05, ***p*<0.01.

ing the adverse effects.

Angelica gigas is an herbal medicinal plant and inhabits the East Asian area like Korea, Japan and China. The roots of the herbal plant have been traditionally used as dietary supplements for pain relief and improvement of gynecological health (Choi *et al*., 2012). The therapeutic value of the ethanol extracts of the roots were examined for several disease conditions including cancer and inflammation (Jiang *et al*., 2007; Kim *et al*., 2015). It was reported that *A. gigas* extracts attenuated the induction of nitric oxide, MCP-1 and TNF-α in macrophage cell lines, suggesting an immunomodulatory activity of the herbal compounds (Kim *et al*., 2006; Cho *et al*., 2015). However, the effect of *A. gigas* on the helper T cell responses has not been determined as well as the anti-inflammatory component of the herbal plant. In this study, we have identified decursinol angelate as an immunosuppressive ingredient of *A. gigas* and investigated its activity on Th17 cell differentiation and function.

MATERIALS AND METHODS

Chemicals and reagents

Dextran sodium sulfate was purchased from Sigma-Aldrich (MO, USA). The chemical compounds of *A. gigas* were provided by Yoongho Lim (Konkuk University, Seoul, Korea): twelve active ingredients of *A. gigas* was purchased from Biopurify Phytochemicals Ltd (Sichuan, China) and the purity of each compound was at least 98% as determined by HPLC system fitted with a RP-C18 column (Phenomenex, Torrance, CA, USA) (Maharjan *et al*., 2018). Fluorophore conjugated antibodies against CD4, CD11b, Gr-1 and IFN-γ; the neutralizing antibodies against IL-4, IL-12 and IFN-γ; the antibody against CD3ε and CD28 were purchased from BD biosciences (CA, USA). FITC conjugated IL-17A antibody was obtained from Thermo Fisher Scientific (MA, USA). Recombinant murine IL-6, IL-23 and human TGF-β were purchased from PeproTech (NJ, USA).

Mice and colitis model

OT-II mice were provided by Mark Boothby (Vanderbilt University, Nashville, TN, USA) and C57BL/6J mice purchased from DBL (Seoul, Korea). All the animal experiments were performed under protocols approved by the Institutional Animal Care and Use Committee of Hallym University (Chuncheon, Korea). 7- to 8-week-old C57BL/6J mice were administered 2.5% DSS through their drinking water for 7 days. Decursinol angelate were intraperitoneally injected at doses of 0.4 mg/kg and 4 mg/kg every other days from day -1 to day 7. The disease activity index was scored as follows: weight loss ('0': no loss; '1': loss >1-5%; '2': loss 5 >10%; '3': loss >10-15; '4': loss >15-20%); presence of blood ('0': absent; '1': present) and stool consistency ('0': firm; '1': loose; '2': diarrhea). Scores were added to give a maximum score of 7.

$CD4^+$ T cell activation and helper T cell culture

A single cell suspension was isolated from spleen and lymph nodes of the mice as previously described (Lee *et al*., 2013). Lymph node cells of the OT-II mice were activated with 0.2 μg/ml $OVA_{323-339}$ peptide in Iscove's Modified Dulbecco's medium containing 10% FBS and cultured with 4 ng/ml IL-2 for 5 days. $CD4^+$ T cells were purified from the spleen of C57BL/6J mice using CD4 microbeads (Miltenyi Biotech, Gladbach, Germany) in accordance with the manufacturer's instructions. CD4 T cells were activated with anti-CD3ε (2.5 μg/ml) and anti-CD28 (2.5 μg/ml) and cultured for 5 days with mitomycin C-treated splenocytes. Th17 culture was supplemented with 5 ng/ml TGF-β, 10 ng/ml IL-6, 10 ng/ml IL-23, 5 μg/ml anti-IL-12, 5 μg/ml anti-IFN-γ and 5 μg/ml anti-IL-4.

Flow cytometry and cytokine beads array (CBA)

Immunofluorescent staining was conducted as previously described (Lee *et al*., 2010). For intracellular cytokine staining, cells were stimulated with 50 ng/ml PMA and 1 μg/ml ionomycin for 6 h in the presence of the Golgi-stop reagent (BD Biosciences). After they were fixed with 4% paraformaldehyde and permeabilized with 1% saponin, the cells were intracellularly stained with FITC-conjugated anti-IL17A, PE-conjugated anti-IL-4, and APC-conjugated anti-IFN-γ antibodies. Data were acquired with the FACS Canto II instrument (BD Biosciences) and analyzed with the FlowJo V10 software. Cytokine levels were measured by the BD CBA Mouse Th1/Th2/Th17 Cytokine kit (BD Biosciences) in accordance with the manufacturer's instructions.

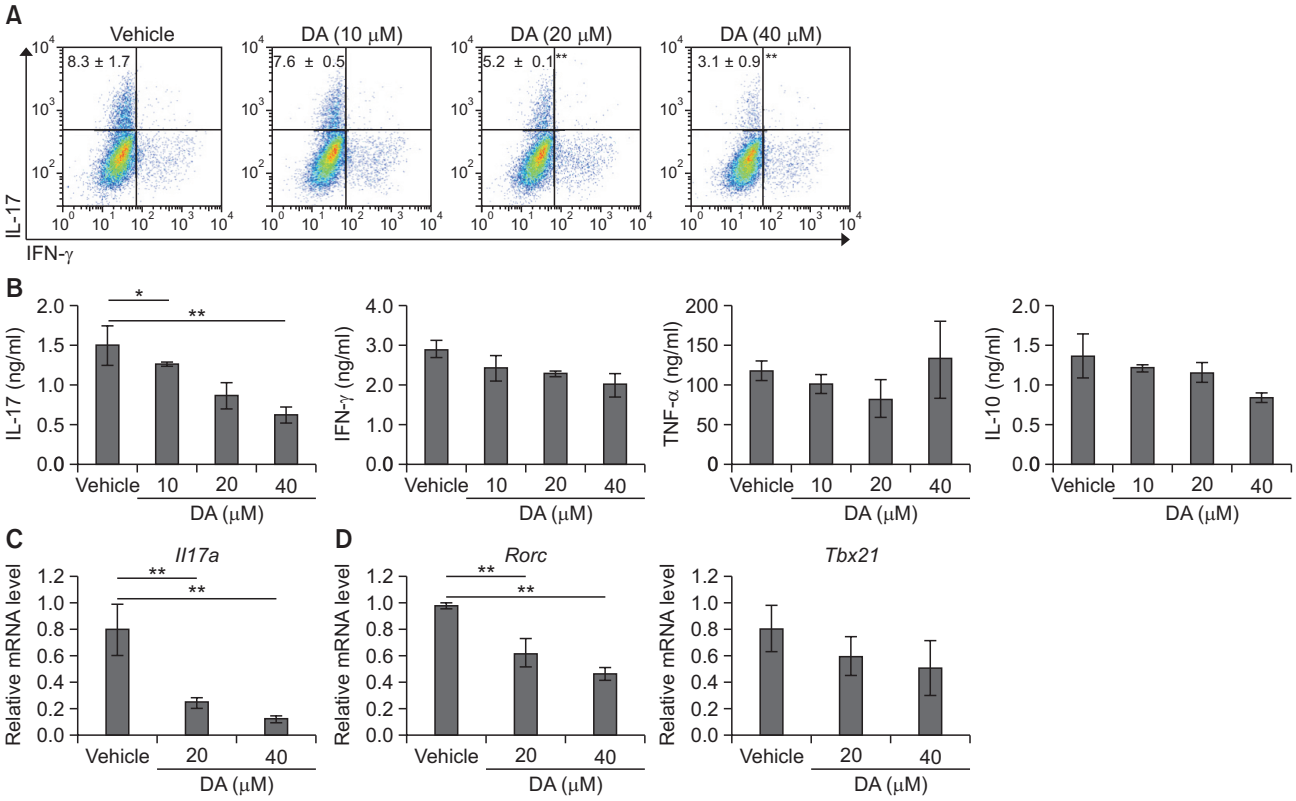

Fig. 2. DA suppresses Th17 differentiation and function. $CD4^+$ T cells were activated and cultured in the presence of DA under Th17 polarizing condition for 5 days. (A) Cells were analyzed by intracellular cytokine staining. Shown are representative FACS profiles in the live CD4 T cell gate: inset numbers indicate the mean (± SD) percentages of $IL\text{-}17^+$ cells obtained from three independent experiments. (B) Cells were restimulated with anti-CD3e and anti-CD28 for 24 h, and the culture supernatants were analyzed by CBA. (C, D) Th17 cells were cultured as in (A) and analyzed by quantitative RT-PCR. Concentrations of the indicated mRNAs were normalized to those of *Actb*, and the means (± SD) of the relative levels from three biological replicates are shown: **p*<0.05, ***p*<0.01.

Cell viability, apoptosis, and proliferation assay

Cell viability assay was performed with the EZ-Cytox Enhanced Cell Viability Assay kit (DoGenBio, Seoul, Korea) in accordance with the manufacturer's instructions. Apoptosis was measured by incubating cells with PE-conjugated Annexin V (BD Biosciences) for 30 min. followed by FACS analysis. $CD4^+$ T cells were activated with anti-CD3ε and anti-CD28 for 3 days, and proliferation was assessed by EdU uptake assay with the Click-iT Plus EdU Flow Cytometry Assay Kit (Thermo Fisher Scientific) in accordance with the manufacturer's instructions.

Quantitative reverse transcription PCR

RNA was extracted using the TRIzol reagent (Thermo Fisher Scientific) and reverse-transcribed into cDNA with M-MLV reverse transcriptase (Promega, WI, USA). Quantitative real-time PCR reaction was performed using a CFX96 Real-Time PCR Detection System (Bio-Rad, CA, USA) with SYBR qPCR mix (Toyobo, Japan). Primer pairs are listed in Supplementary Table 1.

Histology

Colon tissue sections were stained with hematoxylin and eosin staining reagents, and histological scores (0-6) were determined under a microscope as described elsewhere (Longhi *et al*., 2017). The presence of cell infiltration ('0': absence; '1': scattered inflammatory foci; multiple inflammatory foci, and '3': transmural cell infiltration); crypt structure ('0': normal; '1': presence of elongated crypts, and '2': absence of crypts); and edema in the colon wall ('0': absence and '1': presence of edema) were determined.

Cell isolation from the colon

The colons were washed with cold PBS and inverted followed by shaking at 37°C in the presence of 0.015% DTT and 1 μM EDTA for 30 min. The supernatant contained the intraepithelial (colonic IE) cells. The remaining tissue was cut into pieces and incubated at 37°C in 1.5 mg/ml collagenase and 0.5 mg/ml dispase with shaking. The cell suspension containing lamina propria (colonic LP) cells were then washed, passed through a 70 μm strainer, and analyzed by flow cytometry.

Statistical analysis

Experiments were performed in at least duplicate or triplicate, and the results of more than three independent experiments are represented as the mean (± SD) unless mentioned otherwise. The statistical significance between the samples was calculated by unpaired two-tailed *t*-test (Instat; GraphPad Inc, San Diego, CA, USA). In the figures, * and ** denote *p*-

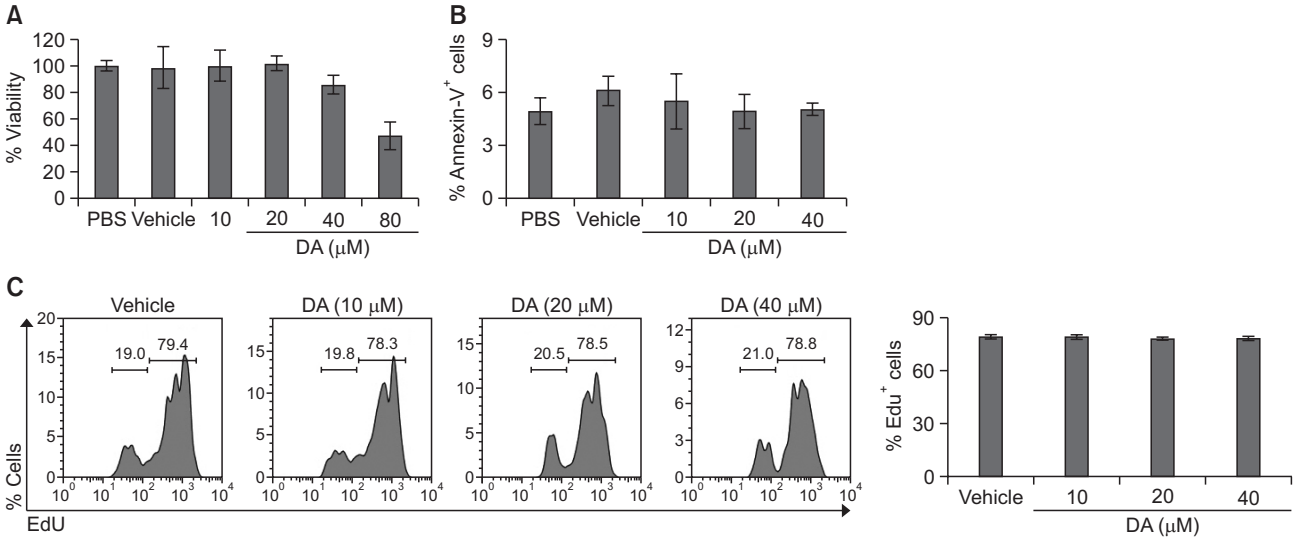

Fig. 3. Effect of DA on survival and proliferation of CD T cells. CD4 T cells were activated with anti-CD3 and anti-CD28 for 3 days in the presence or absence of DA. Cell viability (A) was determine with the Ez-Cytox kit, and apoptosis (B) was measured by Annexin-V staining. The data represent the means (± SD) of three independent experiments. (C) CD4 T cells were cultured as in (A) and analyzed by flow cytometry after a pulse with EdU. Shown are representative histograms in the live CD4 T cell gates and the means (± SD) of EdU$^+$ cells averaging three independent experiments.

values less than 0.05 and 0.01, respectively.

RESULTS

Decursinol angelate as an anti-inflammatory component of *A. gigas* suppresses the proinflammatory cytokine production of CD4 T cells

A. gigas has been used in traditional Korean medicine as a tonic and as a treatment for various diseases including inflammatory conditions (Choi *et al*., 2012). To define the bioactive ingredient of the herbal plant with an immunomodulatory activity, we tested the effects of the chemical components of *A. gigas* on T cell activation using class II-MHC restricted and ovalbumin-specific OT-II T cells. Treatment with several compounds potently attenuated the secretion of proinflammatory cytokines such as TNF-α and IFN-γ from the antigen-stimulated OT-II cells as measured by CBA (Fig. 1A, 1B). Among the tested compounds, decursinol angelate (DA) was able to significantly suppress the IL-17 production of CD4 T cells (Fig. 1C). Next, we further investigated the immunomodulatory activity of DA on differentiation and function of helper T cells.

Decursinol angelate impedes Th17 cell differentiation and IL-17 expression of CD4 T cells

Naïve T cells can be activated and differentiated into distinct subsets of helper T cells including IFN-γ-producing Th1 cells, IL-4-producing Th2 cells, and IL-17-producing Th17 cells to elicit an appropriate adaptive immunity (Zhu and Paul, 2008). To assess the immunomodulatory activity of DA on the helper T cell subsets, we cultured CD4 T cells in the presence of DA under Th1, Th2, and Th17 cell polarizing conditions. Flow cytometry analyses showed that the DA treatment resulted in decreased intracellular expression of IL-17 in the Th17-polarized cells in a dose dependent manner (Fig. 2A). DA also inhibited the expression of IFN-γ in Th1 cells but did not affect that of IL-4 in Th2 cells (Supplementary Fig. 1A, 1B). The CBA results using the culture supernatants confirmed that DA significantly suppressed IL-17 secretion from the activated Th17 cells (Fig. 2B). Moreover, we observed a decreased IL-17A mRNA expression in the DA-treated Th17 cells, which suggests that DA inhibits the induction of IL-17 at the transcriptional level (Fig. 2C). Although DA attenuated the induction of IFN-γ and TNF-α in antigen-stimulated OT-II T cells and Th1 cells (Fig. 1, Supplementary Fig. 2), DA did not affect the production of those cytokines from the Th17 cultures (Fig. 2B). RORγt is the master transcriptional regulator of Th17 development of CD4 T cells, while the transcription factor T-bet promotes Th1 differentiation (Ivanov *et al*., 2006). The mRNA level of *Rorc* (encoding RORγt) was significantly attenuated by the DA treatment in the Th17 cells, but that of *Tbx21* (encoding T-bet) was not affected (Fig. 2D). These results imply that DA negatively modulates the programming of Th17 cells, leading to the impaired production of IL-17.

FoxP3-expressing regulatory T (Treg) cells have a pivotal role in the peripheral tolerance (Rudensky, 2011). Intriguingly, Th17 and Treg cells share a common signaling pathway mediated by TGF-β, and the balance between the Th17 and Treg cells is critical in inflammatory and autoimmune diseases (Lee, 2018). When we measured the anti-inflammatory cytokine IL-10 secreted from the Th17 cultures, we did not observe an enhanced production of IL-10 by the DA treatment (Fig. 2B). Even under the Treg polarizing condition, DA did not affect the induction of FoxP3$^+$ Treg cells (Supplementary Fig. 1C). These results suggest that the anti-inflammatory activity of DA on Th17 cells was not due to a secondary effect on the immunomodulatory function of Treg cells.

Effects of decursinol angelate on the survival and proliferation of CD4 T cells

Previous results demonstrated that DA potently prevented the induction of Th17 cells from naïve CD4 T cells. This anti-in-

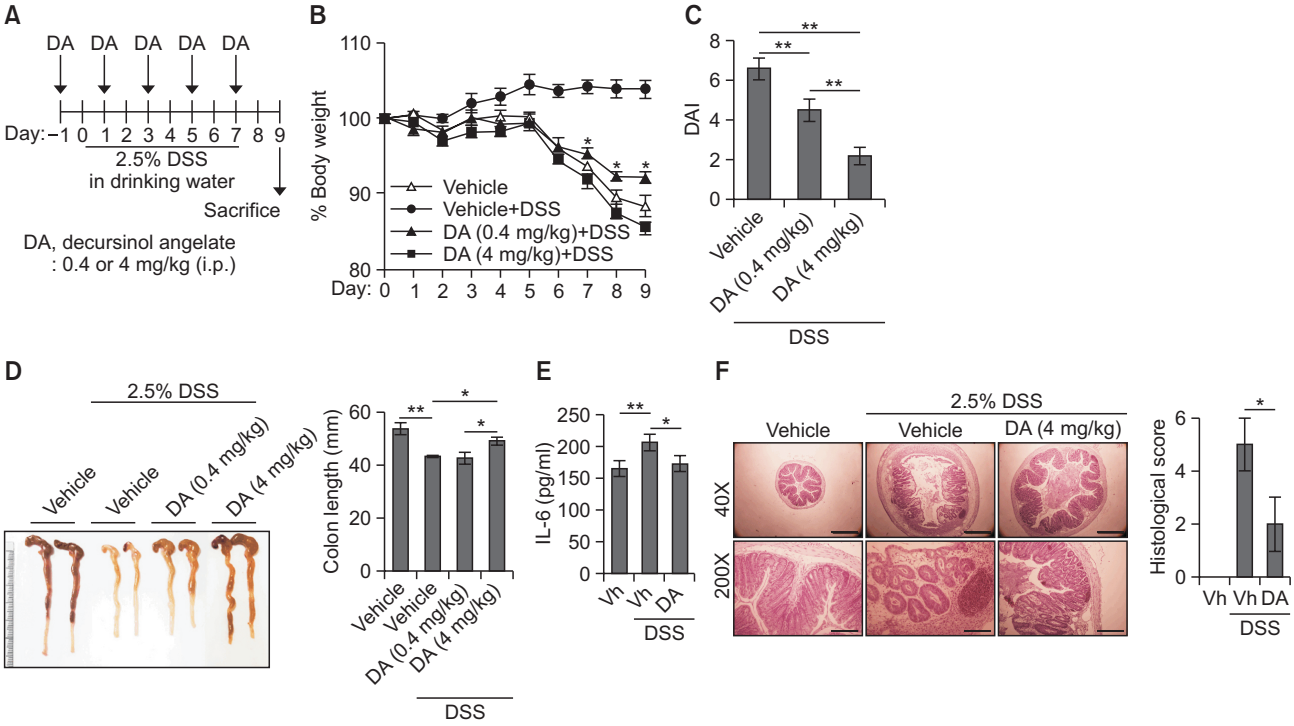

Fig. 4. DA ameliorates DSS-induced colitis in mice. (A) Schematic diagram of experimental procedure. (B) Body weight was measured daily and represented as a mean (± SEM) percentage of body weight. (C) Disease activity index (DAI) was scored as describe in "Materials and Methods". Shown are the means (± SEM) of DAI 9 d after starting DSS administration. Results are obtained from two separate replicate experiments, each involving six mice. (D) At day 9, mice were euthanized, and the colons were isolated and photographed. Colon length was measured. (E) IL-6 level in the plasma at day 9 was analyzed by CBA. (F) Colon were stained with hematoxylin and eosin (H&E). Shown are the representative H&E-stained sections and means (± SD) of the histological scores. **p*<0.05, ***p*<0.01.

flammatory effect of DA would be derived from attenuating the expansion of activated CD4 T cells and/or from cytotoxicity. DA, however, did not affect the viability of CD4 T cells up to a concentration of 40 μM which we used in the previous experiments (Fig. 3A). The Annexin-V staining results also showed that DA did not induce apoptosis of the T cells (Fig. 3B). By using the EdU uptake assay, we measured the effect of DA on cell cycling of activated CD4 T cells in Th17 cultures. Fig. 3C shows that the rate of EdU^+ cells was not reduced by the DA treatment, demonstrating that DA suppresses the differentiation and cytokine production of Th17 cells, independent of the survival and proliferation of CD4 T cells.

Decursinol angelate ameliorates dextran sodium sulfate-induced colitis

Deregulated Th17 responses have been implicated in the pathogenesis of many inflammatory conditions and autoimmune diseases such as IBD (Monteleone *et al*., 2012). To explore whether DA has a therapeutic potential to treat IBD, we used an UC model induced by DSS. Mice given DSS in the drinking water for 7 days exhibited UC symptoms involving weight loss and disease severity that included diarrhea (Fig. 4A-4C). When mice were treated with DA at a dose of 4 mg/kg, the weight loss and colitis severity triggered by the administration of DSS were significantly ameliorated (Fig. 4B, 4C). We also observed that the length of the colon and size of the cecum in the DA-treated mice were longer and bigger than in the vehicle-treated mice, respectively (Fig. 4D). Despite the relatively modest effect with a lower dose (0.4 mg/kg) of DA on the disease severity, the cecum size was bigger than that of the vehicle-treated control (Fig. 4B-4D).

DA treatment significantly suppressed the colitis-induced upregulation of plasma IL-6, as an indicator of systemic inflammation (Fig. 4E). Histological evaluation of the colon sections showed that DSS administration resulted in the histopathology of colitis including muscle thickening, impaired crypt structure, cellular infiltration, and disruption of the epithelium architecture (Fig. 4F). In sharp contrast, the DA treatment protected the mice from the DSS-induced histopathological changes and immune cell infiltration within the colon (Fig. 4F). These results provide the evidence that DA could ameliorate the symptoms and severity of DSS-induced colitis in mice.

Decursinol angelate suppresses the activation of Th17 cells and recruitment of neutrophils in the colitis tissue

To evaluate the inhibitory effect of DA on Th17 responses *in vivo*, we isolated and characterized immune cells in the draining lymph nodes and epithelium of the colons in DSS-administrated mice. IL-17-producing Th17 cells were markedly increased in the mesenteric lymph nodes, colonic intraepithelium and lamina propria by inducing colitis, while IFN-γ-producing Th1 cells were upregulated only in the lamina propria (Fig. 5A). Intriguingly, the DA treatment significantly reduced the induction of Th17 cells in the draining lymph nodes and colonic tissues (Fig. 5A). On the other hand, DA did not inhibit the DSS-triggered IFN-γ-producing Th1 cells in the coli-

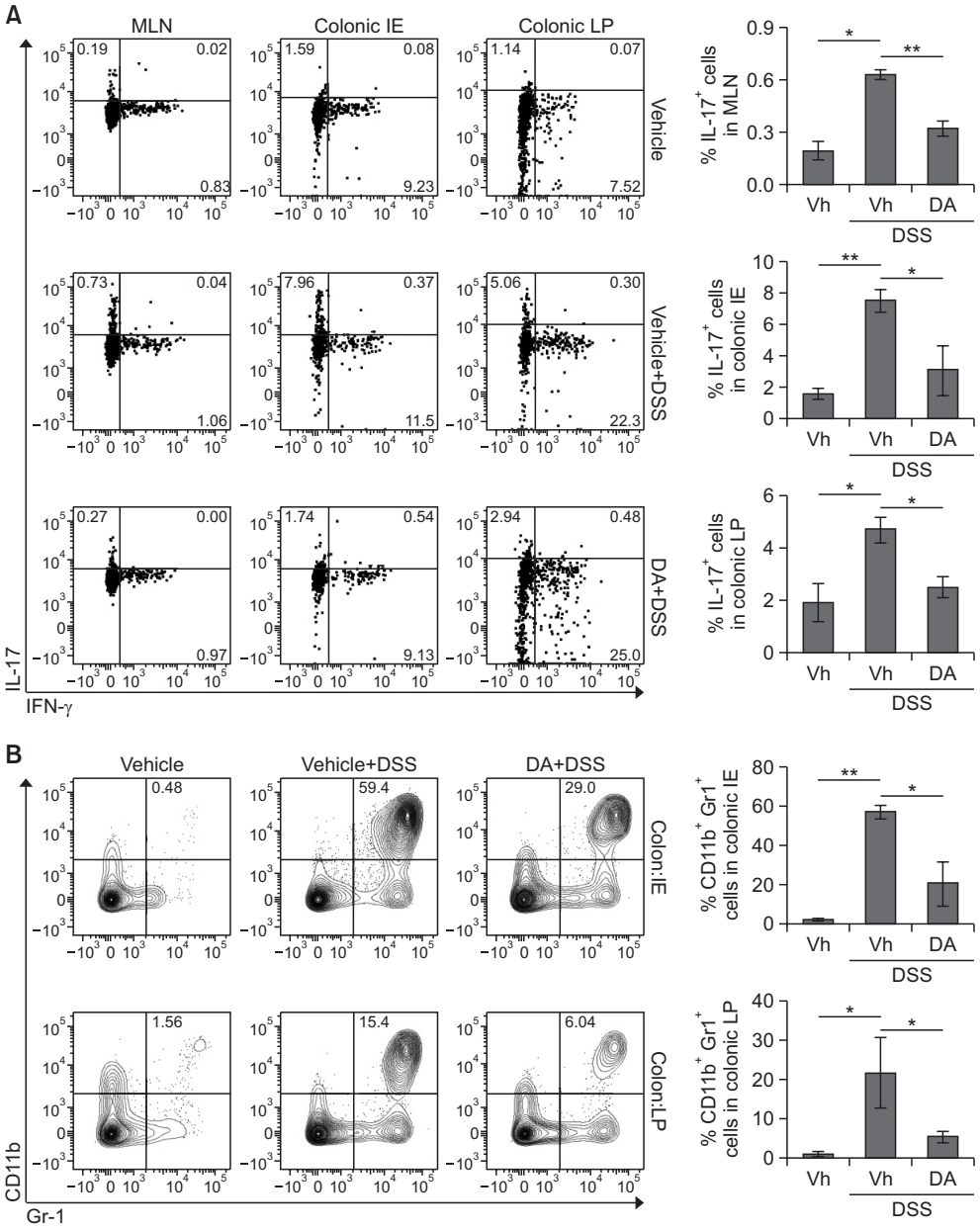

Fig. 5. DA attenuates the activation of Th17 cells and the recruitment of neutrophils in the colitis tissues. (A) Mice were administered with DSS as in Fig. 4, and leukocytes isolate from the mesenteric lymph node (MLN), colonic intraepithelium (colonic IE) and lamina propria (colonic LP) were analyzed by intracellular cytokine staining. Shown are the representative FACS profiles with the inset numbers indicating the prevalence of the IL-17$^+$ and/or IFN-γ^+ cells in the live CD4 T cell gate. The mean (± SD) percentages of the IL-17A$^+$ cells were calculated from two independent experiments, each involving four mice. (B) Infiltration of granulocyte populations in the colonic tissues were analyzed by flow cytometry. Shown are the representative FACS profiles in the live leukocyte gate and mean (± SD) prevalence of CD11b^{hi} Gr-1^{hi}. *p<0.05, **p<0.01.

tis mice. Consistent with the *in vitro* data (Fig. 2), these results confirm the immunomodulatory potential of DA on the activation and function of Th17 responses.

Proinflammatory cytokine IL-17 upregulates a number of cytokines and chemokines in inflamed tissues, leading to the recruitment of neutrophils (Tesmer *et al*., 2008). We observed a dramatic colitis-induced increase of CD11b$^+$ Gr-1$^+$ cells in the colonic tissues (Fig. 5B). In agreement with the reduced induction of Th17 cells, the DA treatment significantly attenuated the recruitment of CD11b^{hi}Gr-1^{hi} neutrophil populations in the colonic tissues of the colitis mice (Fig. 5B). Anti-inflammatory Treg cells were also increased within the draining lymph nodes and colonic tissues upon DSS administration. Although DA did not affect FoxP3 induction and IL-10 production in Treg cells *in vitro*, the DA treatment significantly reduced the prevalence of Treg cells in the colonic tissues of colitis mice (Supplementary Fig. 2). Overall, our data suggest that DA ameliorates DSS-induced colitis through the regulation of

Th17 cells, which contribute to the recruitment of neutrophils in the colonic tissues.

DISCUSSION

The herbal plant *A. gigas* is known as "female ginseng" because it has been used for gynecological health in Korean traditional medicine (Choi *et al*., 2012). The roots of the herbal plant have been reported to have an ability to reduce inflammation. However, most of the previous works have been only *in vitro* studies using macrophage cells lines, but the anti-inflammatory properties on adaptive immune responses were still unknown (Kim *et al*., 2006; Cho *et al*., 2015). In this study, we screened for the bioactive components of *A. gigas* using primary T cells and defined several chemical compounds with an immunomodulatory effect on helper T cell responses. Among them, decursinol angelate specifically suppressed Th17 cells and IL-17 production. As far as we know, this is the first study to reveal that DA, an active ingredient of *A. gigas*, negatively regulates Th17 responses. The DA treatment reduced mRNA expression of RORγt, the master transcription factor for Th17 cell development, while it did not induce apoptosis or attenuate cell cycling at the concentrations that were used in this study. These results suggest that DA is an anti-inflammatory agent regulating Th17 cell differentiation without any cytotoxic or cytostatic effect.

Th17 cells are responsible for protection against extracellular pathogens like fungi and bacteria (Tesmer *et al*., 2008). On the other hand, peripheral tolerance elicited by Treg cells is vital to maintain tolerance, especially in the mucosa of the gastrointestinal tract (Harrison and Powrie, 2013). Otherwise, aberrant activation of Th17 cells leads to the pathogenesis of autoimmune diseases including inflammatory bowel disease (Tesmer *et al*., 2008). Thus, targeting Th17 responses are of particular interest to treat chronic inflammatory conditions. Because DA suppressed Th17 cell differentiation and IL-17 production *in vitro*, we further explored the therapeutic potential of DA on IBD. Various classes of immune cells including innate immune cells and effector $CD4^+$ T cells have been shown to be involved in the induction and establishment of IBD (Matricon *et al*., 2010). Recently, it has been established that Th17 is a key regulator of the pathogenesis of IBD. In fact, IL-17A knockout mice showed milder symptoms in the colon and increased survival when treated with DSS (Ito *et al*., 2008). IL-21, a cytokine secreted by Th17 cells, is upregulated in the mucosa and LP mononuclear cells of patients with IBD, and is reported to enhance the production of metalloproteinases and IFN-γ, which could lead to tissue damage of the intestinal barrier (Monteleone *et al*., 2012). Therefore, regulation of Th17 immune responses has been intensively studied to find a new therapeutic target for the treatment of IBD (Jiang *et al*., 2018).

DSS administered mice exhibit human IBD symptoms such as drastic weight loss, bloody diarrhea, shortening of the colon length and disruption of the colon architecture. When the mice were treated with DA, it significantly ameliorated the IBD symptoms although it did not completely block the progression of the disease (Fig. 4). Consistent with a recent study showing the protective effect of the ethanol extract of *A. gigas* on colitis (Oh *et al*., 2017), our data demonstrate that DA is a bioactive component of the herbal plants with a therapeutic potential to manage IBD. In addition to the protective effect of DA on DSS-induced colitis, we observed substantially reduced plasma level of IL-6 and infiltration of inflammatory cells in the colon tissue. Further, flow cytometry analysis revealed that DA suppressed induction of Th17 cells in the colonic tissues and draining lymph nodes of the DSS-treated mice. Of note, the major inflammatory population in the colitis tissue was $CD11b^{hi}$ $Gr1^{hi}$ neutrophils (~60%), which were significantly decreased by the DA treatment (~15%). In fact, IL-17, the signature cytokine produced by Th17 cells, triggers epithelial cells to secret C-X-C chemokines, which lead to the recruitment of neutrophils to the inflamed tissue (Tesmer *et al*., 2008). In agreement with the Th17 culture data, these results indicate that the reduced infiltration of neutrophils could be partially due to the suppression of Th17 responses. However, it remains to be determined whether DA affects the migration of neutrophils or the chemokine expression of the epithelium.

Treg cells have an immunosuppressive activity by modulating effector functions of T cells and dendritic cells, which is essential for mucosal tolerance to commensal bacteria (Allez and Mayer, 2004). DSS administration not only induced the colitis but also increased prevalence of $FoxP3^+$ Treg cells in the inflamed tissues and draining lymph nodes as a homeostatic mechanism (Supplementary Fig. 2; Boschetti *et al*., 2017). It was intriguing that DA treatment reduced the prevalence of Treg cells in the colitis tissues along with attenuated colonic inflammation and reduced $IL\text{-}17^+$ Th17 cells. On the other hand, *ex vivo* T cell culture experiments revealed that DSS did not affect induction of Treg cells (Supplementary Fig. 1C). Thus, we speculate that the decreased Th17-associated inflammation in the DA-treated mice was not due to reciprocal upregulation of Treg cells.

In summary, our study revealed the immunomodulatory properties of decursinol angelate shown by the suppression of Th17 cell differentiation and function and by the amelioration of DSS-induced colitis in mice. Based on our observations, we propose DA as a novel therapeutic agent for the management of inflammatory bowel disease. Further studies are needed to determine the mechanistic target of DA in the regulation of Th17 cells.

CONFLICT OF INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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