Original Article

Ursolic acid ameliorates autoimmune arthritis via suppression of Th17 and B cell differentiation

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Aim: Ursolic acid (UA) is a pentacyclic triterpenoid found in most plant species, which has been shown anti-inflammatory and antioxidative activities. In this study, we examined the effects of UA on collagen-induced arthritis (CIA) in mice, and to identify the mechanisms underlying the effects.

Methods: CIA was induced in mice. Two weeks later, the mice were treated with UA (150 mg/kg, ip, 3 times per week) for 4 weeks. The expression of cytokines and oxidative stress markers in joint tissues was measured with immunohistochemistry. The numbers of CD4+IL-17+, CD4+CD25+Foxp3+ and pSTAT3 cells in spleens were determined using confocal immunostaining or flowcytometric analyses. Serum antibody levels and B cell-associated marker mRNAs were analyzed with ELISAs and qRT-PCR, respectively. CD4+ T cells and CD19+B cells were purified from mice spleens for in vitro studies.

Results: UA treatment significantly reduced the incidence and severity of CIA-induced arthritis, accompanied by decreased expression of proinflammatory cytokines (TNF-α, IL-1β, IL-6, IL-21 and IL-17) and oxidative stress markers (nitrotyrosine and iNOS) in arthritic joints. In CIA mice, UA treatment significantly decreased the number of Th17 cells, while increased the number of Treg cells in the spleens, which was consistent with decreased expression of pSTAT3, along with IL-17 and RORγt in the splenocytes. In addition, UA treatment significantly reduced the serum CII-specific IgG levels in CIA mice. The inhibitory effects of UA on Th17 cells were confirmed in an in vitro model of Th17 differentiation. Furthermore, UA dose-dependently suppressed the expression of B cell-associated markers Bcl-6, Blimp1 and AID mRNAs in purified CD19+B cells pretreated with IL-21 or LPS in vitro.

Conclusion: UA treatment significantly ameliorates CIA in mice via suppression of Th17 and differentiation. By targeting pathogenic Th17 cells and autoantibody production, UA may be useful for the treatment of autoimmune arthritis and other Th17-related diseases.

Keywords: ursolic acid; rheumatoid arthritis; collagen-induced arthritis; Th17 cell; regulatory T cell; B cell; spleen; proinflammatory cytokine; STAT3

Introduction
Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic joint inflammation that can lead to destruction of the adjacent cartilage and bone. Although the exact molecular mechanisms underlying RA pathogenesis have yet to be elucidated, the current understanding defines RA as a T cell-mediated disease driven primarily by interleukin (IL)-17-producing helper T cells (Th17)1. IL-17 is abundantly expressed in arthritic synovium, and it induces numerous proinflammatory cytokines, including tumour necrosis factor (TNF)-α and IL-1β. Th17 also contributes to antibody production by B cells3 and enhances osteoclastogenesis by upregulating receptor activator nuclear kappa ligand expression4, resulting in bone erosion in arthritic joints. Accordingly, Th17 cells have emerged as a promising therapeutic target in RA, with inhibition of these cells sufficient to ameliorate many of the symptoms of RA.

The phosphorylation of signal transducer and activator of transcription (STAT)3 by Janus kinase (JAK) is a critical process in Th17 cell differentiation5. Upon phosphorylation, STAT3 forms a dimer that is translocated to the nucleus, where it regulates the transcription of its corresponding gene, as well as that of IL-17 and retinoic orphan receptor (ROR) γt, the primary transcription factor controlling Th17 differentiation. Modulation of the JAK-STAT signalling pathway is therefore an effective approach for regulating pathogenic
ultimately in human multiple myeloma cells [16]. This study suggests the STAT3 pathway, leading to the suppression of prolifera-

Of note, Pathak and anti-inflammatory UA has been shown to exert anti-cancer [9], anti-oxidative [10], and anti-inflammatory [11] effects, in addition to conferring benefits in cognitive function [12], asthma [13], and colon cancer [14, 15]. Of note, Pathak et al reported that UA inhibited activation of the STAT3 pathway, leading to the suppression of proliferation in human multiple myeloma cells [16]. This study suggests that UA also acts as an inhibitor of STAT3 activation in T cells, resulting in the suppression of Th17 differentiation. We therefore sought to examine the effects of UA on pathogenic Th17 responses in a CIA model of arthritis.

Materials and methods

Induction of CIA and treatment with UA

Bovine Type II collagen (CII, Chondrex, WA, USA) was dis-
solved overnight in 0.1 mol/L acetic acid (4 mg/mL) with gentle rotation at 4 °C. Eight-week-old male DBA/1J mice (Orientbio, Sungnam, Korea) were injected intradermally at the base of the tail with 100 μg of CII emulsified in complete Freund’s adjuvant (Chondrex). To assess the influence of UA on symptom severity in the CIA model, mice were treated with UA (150 mg/kg) in 10% dimethyl sulfoxide or with vehicle alone by intraperitoneal injection three times a week for 4 weeks beginning 14 days after CII treatment.

Assessment of arthritis

The severity of arthritis was determined by three independent observers. The mice were examined twice a week for the onset and severity of joint inflammation for up to 8 weeks after primary immunization. The severity of arthritis was assessed on a scale of 0–4 using the following criteria, as described previously [17]: 0 = No evidence of erythema and swelling, 1 = Erythema and mild swelling confined to the mid-foot (tarsals) or ankle joint, 2 = Erythema and mild swelling extending from the ankle to the mid-foot, 3 = Erythema and moderate swelling extending from the ankle to the metaphalangeal joint, and 4 = Erythema and severe swelling encompass the ankle, foot, and digits. The arthritis score for each mouse was expressed as the sum of the scores for all four limbs. The highest possible arthritis score for a mouse was therefore 16. The mean arthritis index was used to compare the data among the control and experimental groups.

Histology

Mouse joint tissues were fixed in 4% paraformaldehyde, decal-
cified in EDTA bone decalcifier, embedded in paraffin, and sectioned. The sections were stained with haematoxylin and eosin, safranin O, and toluidine blue to detect proteoglycans.

Immunohistochemistry

Mouse joint tissues were fixed in 10% formalin, decalcified in Calci-Clear Rapid bone decalcifier, embedded in paraffin, and sectioned [18]. The sections were deparaffinised using xylene and dehydrated in a gradient of alcohol solutions. Endoge-

uous peroxidase activity was quenched with 3% hydrogen peroxide in methanol. Immunohistochemistry was performed using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA). The tissues were first incubated with primary antibodies against IL-21, IL-17A, IL-6 (Abcam, Cambridge, UK), IL-1β, TNF-α, nitrotyrosine, induced nitric oxide synthase (iNOS), and an isotype control (Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4 °C. The tissues were then incubated with a biotinylated secondary antibody and streptavidin-peroxidase complex for 1 h. The final coloured product was developed using DAB chromogen (Thermo Scientific, Waltham, MA, USA). Finally, the sections were counterstained with haematoxylin and photographed using a photomicroscope (Olympus, Tokyo, Japan).

Measurement of CII-specific antibodies

Blood was drawn from the orbital sinuses of UA- and vehicle-treated mice; sera were stored at -20 °C until use. Micro-titer plates were coated with CII (4 μg/mL in PBS) at 4 °C overnight, followed by a blocking step for 30 min at room temperature. The serum samples were then diluted 1:10 000 in Tris-buffered saline (pH 8.0) containing 1% bovine serum albumin and 0.5% Tween-20, and incubated in the micro-titre plates for 1 h, after which the plates were washed five times. The concentrations of CII-specific IgG, IgG1, and IgG2a were measured using mouse IgG, IgG1, and IgG2a ELISA Quantitation Kits (Bethyl Laboratories, Montgomery, TX, USA), respectively. Absorbance values were determined with an ELISA microplate reader operating at 450 nm.

CD4+ T cell purification and stimulation

CD4+ T cells were purified from the spleens of DBA/1J mice using a CD4+ T cell MACS isolation kit with an AutoMACS separator, according to the manufacturer’s instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). To establish Th17 cell-polarising conditions, splenocytes were stimulated with plate-bound anti-CD3e monoclonal antibody (0.5 μg/mL, BD Biosciences, San Jose, CA, USA), and anti-CD28 monoclonal antibody (1 μg/mL, BD), anti-IFN-γ antibody (2 μg/mL, R&D Systems, Minneapolis, MN, USA), anti-IL-4 antibody (2 μg/mL, R&D Systems), recombinant TGF-β (2 ng/mL, R&D Systems), and recombinant IL-6 (20 ng/mL, R&D Systems) for 72 h. Total RNA was extracted using TRI reagent (Molecu-
lar Research Center, Cincinnati, OH, USA).

**Cytotoxicity**

Total splenocytes (2 × 10^5 cells/well) were seeded on 96-well flat-bottomed plate and stimulated with differential doses of UA and 1 μg/mL of LPS for 24 h. Four hours before the termination of culture, Cell counting kit-8 (Dojindo Molecular Technologies, Rockville, MD, USA) solution was added in the culture for checking the absorbance at 450 nm using microplate reader.

**Flow cytometry**

For intracellular cytokine staining in mice, cells were stimulated with 25 ng/mL phorbol 12 - myristate 13 - acetate (PMA, Sigma, St Louis, MO, USA) and 250 ng/mL ionomycin (Sigma) in the presence of GolgiStop (BD) for 4 h. The following antibodies were used for intracellular staining: anti-CD4-PerCP, -CD25-APC, -IL-17A-FITC, and -FoxP3-PE antibodies (all eBioscience, San Diego, CA, USA). Events were recorded and analysed with FlowJo software (Tree Star, Ashland, OR, USA).

**Confocal microscopy**

Spleen tissues were snap-frozen in liquid nitrogen and stored at -70 °C. Tissue sections (7 μm) were fixed in acetone and stained for the presence of Treg cells using anti-FoxP3-PE, -CD4-PerCP, and -CD25-APC antibodies (BD). To identify Th17 cells, tissue sections were stained with anti-IL-17A-FITC (eBioscience), -CD4-APC (eBioscience), and -pSTAT3-PE (Tyr705 or Ser727; BD) antibodies overnight at 4 °C, and analysed using an LSM 510 Meta confocal microscopy system (Carl Zeiss, Germany). Positive cells were counted visually at a higher magnification by four individuals.

**Real-time PCR**

A LightCycler 480 II instrument (Roche Diagnostics, Basel, Switzerland) was used for PCR amplification and analysis. All reactions were performed with LightCycler480 SYBR Green I Master according to the manufacturer’s instructions. The primer sequence was listed in the following Table 1. All mRNA expression levels were normalised to that of β-actin with lipopolysaccharide (1 μg/mL, Sigma) for an additional 1 h and then stimulated with lipopolysaccharide (1 μg/mL, Sigma) for an additional 96 h. Total RNA was extracted using TRI reagent (Molecular Research Center).

**Statistical analysis**

Statistical analyses were performed using GraphPad Prism (Version 4 for Windows; GraphPad Software, San Diego, CA, USA). When comparing pairs of groups, the Mann-Whitney U test was used for continuous variables, while the chi-square test was used for categorical variables. P < 0.05 was considered statistically significant. Differences in the mean values of various groups were analysed using an ANOVA with a post-hoc test. P < 0.05 (two-tailed) was considered significant.

### Results

**UA suppresses CIA in mice**

CIA mice were used to investigate the effects of UA on autoimmune arthritis. Mice were injected intraperitoneally with 150 mg/kg UA or vehicle control three times a week, beginning 14 d after CIA treatment. UA treatment significantly reduced the incidence and severity of arthritis compared with the vehicle control (Figure 1A).

Histological examinations performed 49 d after the induction of CIA revealed a lower degree of inflammatory cell infiltration and cartilage loss in the ankles of UA-treated mice compared to those of vehicle-treated mice (Figure 1B). Consistent with reduced cartilage damage, the number of TRAP⁺ cells in the joints of the UA-treated mice was lower than that in the vehicle controls (Figure 1B). Next, we examined whether the anti-arthritic effects of UA were associated with changes in the humoral immune response. UA treatment efficiently inhibited the production of CII-specific total IgG, IgG1, and IgG2a in CIA mice (Figure 2), consistent with a role for B cells in the development of CIA.

The anti-inflammatory effects of UA were further demonstrated through immunohistochemical staining of arthritic joints, which revealed decreased expression of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-21, and IL-17 (Figure 3A and 3B).

As oxidative stress is known to exacerbate arthritic inflammation, we also measured the expression of oxidative stress markers. Nitrotyrosine and iNOS were both suppressed by UA, consistent with the known anti-oxidative properties of UA (Figure 3B).

| Target     | Sequence (5′ to 3′) |
|------------|---------------------|
| IL-17A     | Forward CTTCAAGTCAGGGGTGCC |
|            | Reverse GAGCTCATTTTGGGCAAG |
| FoxP3      | Forward GGGCTTCTCCAGGAGACAGA |
|            | Reverse GCTGTATAGCCGTTGTG |
| RORγt      | Forward GTGCTTGGAGCTACCTACTG |
|            | Reverse GTGCAAGAGGGGAGGACAA |
| Blimp1     | Forward GTGCGGAGGCCTTGGGAG |
|            | Reverse TGGGGACACTTTTGGGGTAG |
| Bcl-2      | Forward CTCGGAGGAGGGAGGATAGT |
|            | Reverse ACACGGGCTATTGCACTCGT |
| Aid        | Forward CGTGTTGAAGAGGAGGATAGT |
|            | Reverse CAGTCGAGATGAGCTTAGGAA |
| β-Actin    | Forward GTACGACCAGAGGCGATACAG |
|            | Reverse GATGACGATACGCGCGGTG |

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**Table 1.** Primer list used for this experiment.
UA influences the proportion of Th17 and Treg cells in mice with CIA
As Th17 cells play a central role in the pathogenesis of RA, we hypothesised that the reduced arthritic inflammation in UA-treated CIA mice resulted from Th17 inhibition. To address this issue, we used confocal microscopy to compare the number of CD4⁺IL-17⁺ cells (Th17) in the spleens of either vehicle- or UA-treated CIA mice 49 d after the induction of CIA. We also examined the number of CD4⁺CD25⁺Foxp3⁺ (Treg) cells in the spleens of the mice, as these cells are known to be reciprocally regulated with Th17 cells and suppress autoimmune inflammation. UA-treated mice had significantly

Figure 1. Ursolic acid (UA) suppresses collagen-induced arthritis (CIA). To assess the influence of UA on CIA, DBA/1J mice were treated with UA (150 mg/kg) or vehicle control three times a week for 4 weeks, beginning 14 d after type II collagen (CII) treatment (n=5 mice per group). (A) Arthritic scores and the incidence of arthritis in CIA-induced DBA/1J mice during the experimental period. (B) Representative histological features of the joints of CIA mice following treatment with either UA or vehicle control. Hematoxylin and eosin (H&E), safranin O, toluidine blue, and TRAP staining results are shown (top). The graph depicts the average histology score and number of TRAP⁺ cells per joint (bottom). The data are presented as the mean±SD of 6 joints per group. †P<0.05, ‡P<0.01 relative to the control mice.
lower numbers of Th17 cells, and a higher number of Treg cells than vehicle-treated controls (Figure 4A). The decrease in Th17 cells with UA treatment was consistently observed when the frequency of CD4+IL-17+ cell in ex vivo splenocytes was assessed by flow cytometry (Figure 4B). To confirm our confocal staining results, the mRNA expression of Th17-associated genes, including RORγt, IL-17, and IL-21, was measured in the splenocytes of CIA mice. The expression of these genes was significantly reduced in the splenocytes of UA-treated mice compared with vehicle-treated controls (Figure 4C).

A key step in Th17 differentiation is the phosphorylation of STAT3 via the JAK-STAT pathway. To investigate whether UA suppressed STAT3 phosphorylation, we counted the number of phosphorylated (p)STAT3+ (either at tyrosine [Y]705 or serine [S]727) CD4+ T cells in the spleens of UA-treated and control mice. The UA-treated mice exhibited a marked reduction in the number of pSTAT3+CD4+ T cells relative to the vehicle-treated controls (Figure 4D). Collectively, these data suggest that UA suppresses Th17 production and enhances Treg cell populations in CIA mice via the inhibition of STAT3 phosphorylation.

**UA suppresses Th17 differentiation in vitro**

Next, we assessed whether the inhibitory effects of UA on Th17 cells in the CIA model could be confirmed using an *in vitro* model of Th17 differentiation. CD4+ T cells were isolated from DBA/1J mice and incubated in Th17-skewing conditions (anti-IL-4 Ab, anti-IFN-γ Ab, IL-6, and TGF-β) in the presence or absence of various concentrations of UA. None of the doses used in these experiments was cytotoxic, as represented by cell viability assay using tetrazolium-based cell counting kit-8 (Dojindo) (Figure 5A). Flow cytometry revealed that UA inhibited Th17 cell differentiation in a dose-dependent manner (Figure 5B). Suppression of Th17 cell differentiation was also confirmed via the reduced mRNA expression of IL-17, IL-21, and RORγt (Figure 5C). Interestingly, in contrast to the enhanced Treg population observed in the CIA model, the frequency of Treg cells was not increased by UA treatment *in vitro* (data not shown). Similarly, Foxp3 expression was unaffected by UA (Figure 5C).

**UA represses B cell activation and differentiation in vitro**

UA-mediated reductions in the CII-specific antibody responses observed in the CIA model may be due to reduced numbers of Th17 cells, which function as B cell helpers. To clarify this issue, we examined the direct effects of UA on B cell activation in *in vitro*. First, to confirm that UA reduces antibody production *in vitro*, total splenocytes from DBA/1J mice were stimulated with LPS and then treated with various concentrations of UA. After 4 d, antibody levels were measured in the culture supernatant. UA significantly reduced IgG, IgG1, and IgG2a production, consistent with what had been seen *in vivo* (Figure 6A). Next, to investigate the direct effects of UA on B cells, CD19+ B cells were isolated from DBA/1J splenocytes and pre-treated with various concentrations of UA. These cells were then cultured in the presence or absence of LPS or IL-21, and the expression of B cell-associated markers were measured by real time RT-PCR. No cytotoxicity was observed at any of the doses used in these experiments (data not shown). The mRNA expression of B cell lymphoma (Bcl)-6, Blimp1, and activation-induced cytidine deaminase (AID) was diminished following UA treatment (Figure 6B). These data suggest that UA suppresses B cell activation not only through its effects on Th17 cells, but also by directly inhibiting B cell activation and differentiation.

**Discussion**

In this study, we demonstrated that UA suppresses STAT3 phosphorylation and Th17 differentiation while enhancing Treg differentiation. UA also repressed B cell activation and differentiation, resulting in decreased antibody production. Together, these results provide a clear mechanistic basis by which UA decreases both the incidence and severity of CIA.

UA confers its anti-inflammatory activities through a number of different mechanisms, including inhibiting the production of proinflammatory cytokines such as IL-2, IFN-γ, and TNF-α, prostaglandin E2, and cyclooxygenase 2. Earlier studies suggested that the anti-inflammatory properties of UA were mediated by the suppression of nuclear factor (NF)-κB, a major transcription factor that regulates the expression of multiple proinflammatory cytokines.

The effects of UA on immune cells have also been add-
Figure 3. UA decreases the expression of proinflammatory cytokines and oxidative stress-related genes in the joints of CIA mice. Ankle joints (6 per group) of CIA mice treated with either UA or vehicle control were immunostained for (A) IL-1β, IL-6, TNF-α, (B) IL-17, IL-21, nitrotyrosine, and iNOS, or (C) isotype control. Representative data are shown.
Figure 4. UA reduces STAT3 phosphorylation and decreases the frequency of Th17 cells within the population of CD4+ T cells in CIA mice. (A) Spleens of UA- or vehicle-treated CIA mice were subjected to immunostaining for CD4+IL-17+, CD4+CD25+, and CD4+Foxp3+. (B) The frequency CD4+IL-17+ cells among ex vivo total splenocytes was assessed using flow cytometry. (C) The mRNA expression of IL-17, IL-21, and RORγt was determined by real-time RT-PCR in cells obtained from the spleen (Sp) or draining lymph nodes (LN). (D) Spleens of UA- or vehicle-treated CIA mice were subjected to immunostaining of CD4+pSTAT3 Y705+ or CD4+pSTAT3 S727+ cells. The data are presented as the mean±SD of 3 animals per group. *P<0.05, **P<0.01.
ressed. The clear association between UA and NF-κB signalling strongly suggested a role for T cell activation. Indeed, Zeng et al demonstrated a dose-dependent decrease in T cell proliferation following UA treatment, as well as reduced IL-2 production\textsuperscript{[22]}. Similarly, UA was suggested to be involved in the suppression of Th1 cytokines (e.g., IL-2 and IFN-γ) in combination with increased Th2 cytokines (e.g., IL-4, IL-5, and IL-10)\textsuperscript{[23]}; however, the mechanism underlying these effects was not clearly shown.

Recently, Xu et al reported that UA suppressed IL-17 production by selectively antagonizing the function of ROR\textgamma\textsuperscript{t}\textsuperscript{[24]}. They demonstrated that UA inhibited the binding of ROR\textgamma\textsuperscript{t} to its coactivator protein, thereby suppressing its transcriptional activity, even though the expression of ROR\textgamma\textsuperscript{t} was unaffected.
They also argued that UA did not affect STAT3 phosphorylation in Th17 cells. In contrast, our data clearly show a reduction in RORγt mRNA expression and the number of pSTAT3-positive cells following UA treatment. This discrepancy may have resulted from the slightly different cytokine cocktails used to induce Th17 differentiation or from differences in the cell types used in these experiments. Indeed, UA has been shown to inhibit STAT3 activation in human multiple myeloma and colon cancer cells. Even though these results were obtained using cancer cell lines, these findings are consistent with our results showing that UA inhibits STAT3 activation.

We observed relative increases in the Treg populations in UA-treated CIA mice in vivo; however, this was not the case in our in vitro experiments. Considering that Foxp3 expression was not affected by UA treatment, it is unlikely that UA

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**Figure 6.** UA negatively affects B cell function in vitro. (A) Total splenocytes were obtained from DBA/1J mice and stimulated with 1 µg/mL LPS and incubated with various concentrations of UA. After 4 d, the levels of total IgG, IgG1, and IgG2a in the supernatant were determined by ELISAs. (B) CD19+ B cells were isolated from the spleens of DBA/1J mice and pre-treated with either 1 µg/mL LPS or 50 ng/mL IL-21, and then cultured with various concentrations of UA. After 4 d, the mRNA expression of Blimp1, Bcl-6, and AID was analysed by real-time RT-PCR. *p<0.05, **p<0.01 relative to the controls.
increased Treg differentiation directly. When differentiation into Th17 cells is suppressed under inflammatory conditions, T cells may instead be converted into Treg cells, especially considering the plasticity of Th17 and Treg cells.[29]

Although RA is largely considered to be a T cell-mediated disease, the humoral immune response, as represented by autoantibody production, plays another key role in the pathogenesis. This prompted us to investigate the direct effects of UA on B cells. LPS- or IL-21-stimulated antibody production was markedly suppressed following UA treatment. We hypothesised that this was due to reduced plasma cell differentiation and isotype switching. Indeed, the expression of Blimp1, a master transcription factor that drives plasma cell differentiation, and AID, an essential enzyme in isotype switching, was markedly decreased following UA treatment. Interestingly, UA also suppressed Bcl-6 expression in these cells, a somewhat contradictory result given that Bcl-6 is known to be repressed during plasma cell differentiation. However, B cells express both Bcl-6 and Blimp1 in response to IL-21 stimulation and differentiate either into germinal centre B cells or plasma cells, respectively, in a context-dependent manner.[27] Therefore, it can be concluded that UA generally inhibits B cell activation and differentiation mediated by IL-21 or LPS.

The inhibitory effects of UA on B cells appear to be mediated by the suppression of pSTAT3, similar to the effects seen in T cells. STAT3 activation was recently shown to play an important role in the differentiation of B and Th17 cells,[28], particularly IL-21-mediated plasma cell differentiation, which is dependent upon pSTAT3.[29] These findings, together with our data showing pSTAT3 suppression by UA, suggest that the reduced expression of B cell differentiation markers resulted from the inhibition of STAT3 phosphorylation. Further investigation will be necessary to clarify whether UA is able to directly inhibit STAT3 phosphorylation in B cells.

From the results presented here, it appears that reduced CII-specific IgG production in vivo occurs as a result of both the direct inhibition of B cells and Th17-mediated B cell activation. Indeed, Th17 cells, which are decreased by UA treatment, are known to play a role in antibody production.[30] These results are consistent with other known functions of STAT3, including the generation of follicular helper T cells[30] and development of Th2-mediated B cells.[31] Taken together, it is clear that UA ameliorates autoimmune arthritis by targeting multiple processes associated with STAT3 activation.

**Conclusion**

UA inhibits Th17 cell proliferation and promotes Treg cell differentiation by inhibiting the expression of RORγt and pSTAT3. UA also inhibits B cell activation and plasma cell differentiation, leading to a reduction in serum antibodies. By targeting pathogenic Th17 cell proliferation and autoantibody production, UA has potential as a novel therapeutic for the treatment of autoimmune arthritis. The consumption of UA-rich foods may help prevent autoimmune arthritis and other Th17-related diseases.

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**Author contribution**

Seung-ye BAEK, Jaeoseon LEE, Dong-gun LEE, Mi-kyung PARK, Jennifer LEE, Seung-ki KWOK, and Sung-hwan PARK contributed to conception and design, or acquisition of data, or analysis and interpretation of data; Seung-ye BAEK, Jaeoseon LEE, Jennifer LEE, Seung-ki KWOK, Mi-la CHO, and Sung-hwan PARK were involved in drafting the article or revising it critically for important intellectual content; Seung-ye BAEK, Jaeoseon LE, Jennifer LEE, Seung-ki KWOK, and Sung-hwan PARK approved the final version to be published.

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