Ginsenoside metabolite compound K suppresses T cell priming via modulation of dendritic cell trafficking and costimulatory signals resulting in alleviation of collagen-induced arthritis

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CK suppresses T cell priming by modulation of dendritic cell

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Non-standard abbreviations:
AA, adjuvant-induced arthritis; APCs, antigen-presenting cells; CFA, complete Freund’s adjuvant; CIA, collagen-induced arthritis; CK, ginsenoside metabolite compound K; DCs, dendritic cells; MTX, methotrexate; RA, rheumatoid arthritis; TCR, T cell receptor.
Abstract

Ginsenoside metabolite compound K (CK, 20-O-D-glucopyranosyl-20(S)-protopanaxadiol), a novel ginsenoside metabolite, belongs to dammarane-type triterpene saponins according to its structure. The anti-inflammatory activity of CK has been identified in several studies. Our study demonstrated that CK exerted anti-inflammatory effect on collagen-induced arthritis (CIA) and adjuvant-induced arthritis (AA) animal models, and this effect was due to inhibiting the abnormal activation and differentiation of T cells. However, the mechanism of CK on suppressing T cell activation remains unclear. In this study, the results show that CK had a therapeutic effect on CIA mice, decreased the percentage of the activated T cells and dendritic cells (DCs), and increased the percentage of naive T cells in lymph nodes. The inhibitory effect on T cell activation of CK was related to suppressing accumulation of DCs in the lymph nodes. CK decreased CCL21 level in lymph nodes and CCR7 expression on DCs and suppressed CCL21-CCR7-mediated migration of DCs, thus reducing accumulation of DCs in lymph nodes. In addition, signals for T cell activation including MHCII and costimulator molecules, such as CD80 and CD86, were suppressed by CK, and the proliferation of T cells induced by DCs was inhibited by CK. In conclusion, this study demonstrated that CK downregulated DC priming T cell activation in CIA, and suppressing
CCL21-CCR7-mediated DC migration and signals between T cell and DC might be the potential mechanisms. These results provide an interesting novel insight into the potential mechanism by which CK contributes to the anti-inflammatory effect in autoimmune conditions.
Introduction

Numerous studies have confirmed the role of T cells in the pathogenesis of autoimmune diseases such as rheumatoid arthritis (RA). The excessive activation of T cells causes undesirable immune responses against self-antigens, which then results in chronic inflammatory and autoimmune response (Rodeghero R et al., 2013; Pablos JL and Cañete JD, 2013). In our previous study, a decreased percentage of naive T cells, a raised percentage of activated T cells, and hyperplasia of T cells were observed in autoimmune arthritis animal models (Zhang L et al., 2013; Song SS et al., 2013; Chang Y et al., 2011; Liu Y et al., 2011).

Ginsenoside metabolite compound K (CK, 20-O-D-glucopyranosyl-20(S)-protopanaxadiol) \( (C_{36}H_{62}O_{8}, \text{MW: 622.88}) \) belongs to dammarane-type triterpene saponins according to its structure (Fig. 1) (Chi H and Ji GE, 2005). It is a degradation product of ginsenoside in the intestine by bacteria and is the major form of ginsenoside absorbed into the body (Tawab MA et al., 2003; Lee J et al., 2009) and can be transformed from ginsenoside by food microorganisms in vitro (Chi H and Ji GE, 2005). The anti-inflammatory activity of CK has been identified in several studies. CK suppresses proinflammatory cytokine production such as TNF-\( \alpha \), IL-12, and IL-6 and inhibits COX-2 expression (Lee ES et al., 2011; Park EK et al., 2005; Yang CS et al., 2008; Joh EH et al., 2011). Our previous study demonstrated that CK
attenuated arthritis in animal models by suppressing T cell activation. CK decreased the percentage of activated T cells; suppressed T cell receptor (TCR), CD28, and CD25 expression on T cells; and inhibited inflammatory cytokine production such as IL-1β, TNF-α, IL-2, and IL-17 (Wu H et al., 2014; Chen J et al., 2014; Liu KK et al., 2014). However, the mechanism of CK on suppressing T cell activation remains unclear.

Activation of T cells requires signals provided by antigen-presenting cells (APCs), including MHCII-TCR and B7 (CD80, CD86)-CD28 (Ganguly D et al., 2013). Dendritic cells (DCs) are the most powerful APCs in the body. DCs have been shown to continuously present endogenous antigens and prime the T cell response in inflammatory conditions (Bailey SL et al., 2007; Leung BP et al., 2002; Eriksson U et al., 2003). The role of DCs in the pathogenesis of autoimmune disease, such as RA, has been confirmed in numerous studies (Rodríguez-Fernández JL, 2013; Miles B et al., 2014; Pulendran B et al., 2010). Studies have demonstrated that the infiltrated DCs in the inflamed synovial tissue subsequently migrate to the draining lymph nodes and present arthritogenic peptide to T cells and induce naive T cell activation (Pettit AR et al., 2000; Lutzky V et al., 2007). The homing of DCs from peripheral to second lymph organs is a basic feature for their ability to prime effective T cell immune response. In inflammation conditions, migration of DCs induces pathogenic T cells such as Th1 and Th17 (Ma
Migration of DCs from peripheral tissues to lymph nodes is induced by the chemokine CCL21 and its receptor CCR7 (Tal O et al., 2011). CCL21 expressed in high endothelial venule is required for antigen-laded mature DCs to migrate to lymphatic vessels, and this has been shown to increase massively during inflammation (Johnson LA and Jackson DG, 2010).

Therefore, in this study, we observed the effect of CK on T cell activation in CIA mice and investigated the mechanism focused on migration of DCs and signals provided by DCs. The results demonstrate that CK downregulated DC priming T cell activation in CIA, and suppressing CCL21/CCR7-mediated DC migration and signals between T cell and DC might be the potential mechanisms.
Materials and methods

Animals

Sixty male DBA/1 mice (18 ± 2g, Shanghai SLAC Laboratory Animal Co., Ltd., China) were used in this study. All mice were maintained in specific pathogen-free animal laboratory of Anhui Medical University. All experiments were approved by the Ethics Review Committee for Animal Experimentation of Institute of Clinical Pharmacology, Anhui Medical University.

Reagents

Complete Freund’s adjuvant (CFA) and chick type II collagen were obtained from Chondrex Inc (USA); RPMI-1640 medium from Gibco Co., Ltd. (USA); CCL21 ELISA kit from Cusabio Biotech Co., Ltd (China); fluorescence-conjugated mAbs to CD4, CD62L, CD25, CD11c, CD80, CD86, and MHCII from Biolegend Co. USA. Anti-CD11c and T cell microbeads were from Miltenyi Co., Ltd. (GER); CCK kit from Dojindo Molecular Technologies, Inc., (USA); and mAb to CCR7 from Abcom Co., Ltd. (USA). CK (purity>98%) was provided by Zhejiang Haizheng Medicine Co., Ltd., and methotrexate (MTX) was obtained from Shanghai Xinyi Pharmaceutical Co., Ltd. (China).

Induction and treatment of CIA

CII was dissolved in 0.1 mol/L acetic acid and emulsified with an equal volume of CFA at 2 mg/mL and then incubated overnight at 4°C. DBA/1
mice were injected intradermally twice with 0.1 mL of this emulsion (100 μg of CII/mouse) at the back and the base of the tail. The day of the first immunization was defined as d 0, and the booster injection was administered into the back on d 21 (Zhang L et al., 2013). Mice were divided into six groups (n=10 per group) including normal, CIA, CK (14 mg/kg), CK (56 mg/kg), CK (224 mg/kg), and MTX (2 mg/kg). After the onset of arthritis at d27, mice were given CK, intragastric administration, once a day, for 21 days (during secondary arthritis phase). MTX (2 mg/kg) was administered intragastrically, once in 3 days, eight times. Meanwhile, the normal and model animals were given an equal volume of normal saline.

**Evaluation of arthritis**

To evaluate the severity of arthritis, arthritis global assessment and swollen joint count of mice were evaluated every 3 days by two observers blinded to the treatment. The arthritis global assessment was based on symptoms in different parts of the CIA mice: ear (0 = no nodule or redness, 1 = nodule and redness on one ear, and 2 = nodules and redness on both ears), nose (0 = no connective tissue swelling or redness and 1 = evident connective tissue swelling and redness), tail (0 = no nodule or redness and 1 = evident nodule and tail redness), and paw (0 = no swelling or redness, 1 = one front or hind paw swelling and redness, 2 = two paws swelling and redness, 3 = three paws swelling and redness, and
4 = four paws swelling and redness). The above-mentioned scores were tallied to form the arthritis global assessment, and a maximum value of each mouse was 8. Each paw has five phalanx joints and one ankle or wrist joint, so the maximum swelling joint count for each mouse was 24 (Wang QT et al., 2013).

**Preparation of mononuclear cells and T cells**

Lymph node single-cell suspension was collected by mechanical dissociation of lymph node through nylon mesh. Mononuclear cells were purified from the gradient interphase. Then, the cells were washed with PBS three times and suspended in RPMI-1640 medium at a concentration of $1 \times 10^7$ cells/L for DC and T-cell subsets assay. Then, CD4+ T cells were purified by MicroBeads (Miltenyi) for mixed lymphocyte reaction assay.

**Culture of bone marrow-derived DC**

Bone marrow-derived DCs were generated from mice bone marrow cells in the presence of rmGM-CSF and rmIL-4 (Song S et al., 2014). On day 7, the dendritic proliferation clusters were collected and purified by anti-CD11c MicroBeads as immature DCs. Purified DCs were stimulated with LPS (100 ng/mL) for 24 h as mature DCs.

**Flow cytometry**
To assay the subsets of T cells and DCs and expression of surface marker (CD80, CD86, MHCII) and CCR7, fluorescence-conjugated antibodies were added to single-cell suspension (100 μl) prepared previously. After mixing gently, the samples were incubated for 20 min at 4°C and then analyzed using flow cytometry.

**CCL21 determination**

Lymph nodes from each group were weighed and homogenized (100 mg tissue per 1 ml of homogenization buffer). The samples were subjected to one round of freeze thaw and then sonicated for 10 minutes. The homogenate was centrifuged at 3000g for 10 min. The supernatants were collected for CCL21 measurements using ELISA.

**CCR7 analysis by Western blot**

DCs prepared previously were lysed in cell lysis buffer, and denatured protein was separated by 10% SDS-PAGE and transferred electrophoretically to PVDF membrane. After incubating with blocking buffer (0.05% Tween 20-PBS with 5% nonfat milk) at 37°C for 2 h, primary antibody to CCR7 was used at a final dilution of 1:2000 for overnight at 4°C. After incubating with the appropriate goat antirabbit antibody at 37°C for 2 h. Immunodetection was carried out using enhanced chemiluminescence reagent according to the manufacturer’s instructions. Equivalent protein loading and transfer efficiency were verified using staining for β-actin.
Transwell assay

Mature DCs (5 × 10^5 cells) were placed in transwell chambers (5.0 μm, Corning) with or without CK (10^{-9}M, 10^{-7}M, 10^{-5}M); culture media 600 μl with CCL21 (50 ng/ml) was added in 24-well plates and then incubated at 37°C with 5% CO₂ for 18 h. Migratory cells into the bottom chamber were determined using cell counter (cellometer; Nexcelom). The migration index was calculated as the number of cells transmigrating with chemokine divided by the number of transmigrating cells in the absence of chemokine (Calpe E et al., 2011).

Mixed lymphocyte reaction

Prepared DCs (5 × 10^4 cells/well) and T cells (1 × 10^6 cells/well) were placed in 96-well plates at a ratio of 1: 20 and then cocultured at 37°C with 5% CO₂ for 44 h. T cell proliferation was assayed using Cell Counting Kit assay. Ten microliters of CCK was added to each well; the cells were then incubated for 4 h, and the absorbance at 450 nm was recorded using a 96-well plate reader (Zhu YP et al., 2013).

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Statistical significance was determined by One-Sample T Test, where P < 0.05 was considered statistically significant.
Results

CK alleviated the severity of arthritis in CIA mice

To assess the therapeutic effect of CK on CIA mice, the severity of arthritis was evaluated. After onset of arthritis on d27, there was a significant increase in arthritis global assessment and swollen joint count in CIA mice. Administration of CK (56 mg/kg) alleviated the global assessment (d45: \( t = 2.333, P = 0.031 \); d48: \( t = 2.212, P = 0.040 \); d51: \( t = 2.703, P = 0.015 \); Fig. 2A) and swollen joint count (d45: \( t = 2.762, P = 0.013 \); d48: \( t = 2.423, P = 0.026 \); d51: \( t = 2.640, P = 0.023 \); Fig. 2B) significantly when compared with CIA mice.

Administration of CK (224 mg/kg) alleviated the global assessment (d45: \( t = 2.400, P = 0.027 \); d48: \( t = 2.554, P = 0.020 \); d51: \( t = 3.239, P = 0.005 \); Fig. 2A) and swollen joint count (d45: \( t = 2.867, P = 0.010 \); d48: \( t = 3.757, P = 0.001 \); d51: \( t = 4.821, P = 0.001 \); Fig. 2B) significantly when compared with CIA mice.

The effect of CK on the subsets of T cells and DCs in lymph nodes from CIA mice.

To evaluate effect of CK on T cell activation of CIA mice, the subsets of naive T cells and the activated T cells were assayed in CIA lymph nodes. The results show that naive T cells decreased (\( t = -7.432, P = 0.001 \), Fig. 3, A and D), and the activated T cells increased (\( t = 3.552, P = 0.005 \), Fig.
3, B and E) in CIA mice when compared with normal mice. Treatment of CK (56 mg/kg, 224 mg/kg) resulted in an increase of naive T cells (CK 56 mg/kg: \( t = -4.654, P = 0.004 \); CK 224 mg/kg: \( t = -9.613, P = 0.000 \), Fig. 3, A and D) and a decrease of the activated T cells in lymph nodes (CK 56 mg/kg: \( t = 2.763, P = 0.020 \); CK 224 mg/kg: \( t = 3.849, P = 0.009 \), Fig. 3, B and E). Percentage of DCs increased in CIA lymph nodes when compared with normal mice (\( t = 5.442, P = 0.001 \), Fig. 3, C and F). Administration of CK (56 and 224 mg/kg) decreased the percentage of DC (CK 56 mg/kg: \( t = 3.116, P = 0.011 \), CK 224 mg/kg: \( t = 5.737, P = 0.001 \); Fig. 3, C and F) in lymph nodes. Interestingly, percentage of DCs and naive T cells revealed a negative correlation (\( R^2 = 0.8348, P = 0.03 \), Fig. 3G), whereas percentage of DCs and the activated T cells revealed a positive correlation (\( R^2 = 0.9830, P = 0.0009 \), Fig. 3 H).

**CK suppressed DC migration induced by CCL21**

CCL21 is the major chemokine, which mediated DC migration from the periphery to the lymph nodes. To clarify whether the changed percentage of DCs in lymph nodes was associated with migration, a transwell assay was designed; CCL21 level in the lymph nodes and CCR7 expression on DCs were detected. The results show that the migration index of DCs induced by CCL21 was inhibited by CK (100 nM, 10 \( \mu \)M) (CK 100 nM: \( t = 5.356, P = 0.006 \); CK 10 \( \mu \)M: \( t = 8.094, P = 0.001 \); Fig. 4A). CCL21 in the lymph nodes increased in CIA mice when compared with normal
mice \((t = 8.216, P = 0.001, \text{Fig. 4B})\), CK (56, 224 mg/kg) decreased CCL21 level in the lymph nodes (CK 56 mg/kg: \(t = 3.481, P = 0.025\); CK 224 mg/kg: \(t = 6.702, P = 0.003\); Fig. 4B). Interestingly, the percentage of DCs and CCL21 level revealed a positive correlation \((R^2 = 0.7802, P = 0.0470, \text{Fig. 4C})\). In addition, CK (100 nM and 10 \(\mu\)M) decreased CCR7 expression on DCs (CK 100 nM: \(t = 10.119, P = 0.001\); CK 10 \(\mu\)M: \(t = 5.931, P = 0.004\); Fig. 4, D and E) (CK 100 nM: \(t = 9.746, P = 0.001\); CK 10 \(\mu\)M: \(t = 11.540, P = 0.000\); Fig. 4, F and G).

**CK impaired T-cell-stimulatory capability of DCs**

In the next study, T-cell-stimulatory capacity of DCs was assayed by mixed lymphocyte reaction. T cell proliferation was significantly induced by DCs \((t = 6.690, P = 0.001, \text{Fig. 5})\). The ability of DCs on priming T cell proliferation was impaired when DCs were pretreated with CK (100 nM and 10 \(\mu\)M) before co-culture with T cells (CK 100 nM: \(t = 2.707, P = 0.035\); CK 10 \(\mu\)M: \(t = 3.877, P = 0.008\); Fig 5).

**CK impaired CD80, CD86, and MHCII expression on DCs**

CD80 and CD86 are important costimulatory molecules expressed on DCs, and MHCII is the molecule associated with the presenting antigen. They can provide signaling for T cell activation. In the next study, we investigated whether CK affected the expression of these molecules on DCs. After treatment with CK (100 nM and 10 \(\mu\)M) in vitro, expression of CD80 (CK 100 nM: \(t = 3.346, P = 0.015\); CK 10 \(\mu\)M: \(t = 4.211, P =
0.006; Fig. 6, A and B), CD86 (CK 100 nM: \( t = 3.205, P = 0.018 \); CK 10 
μM: \( t = 4.063, P = 0.007 \); Fig. 6, C and D), and MHCII (CK 100 nM: \( t = 
3.174, P = 0.019 \); CK 10 μM: \( t = 3.797, P = 0.009 \); Fig. 6, E and F) on 
DCs were decreased.
Discussion

Numerous studies have confirmed the role of T cells in the pathogenesis of RA. RA has been regarded as a classical T-cell-mediated chronic inflammatory autoimmune disease for years. Abnormal activation of T cells in RA are the main induced factors causing the RA immune damage and synovial pathological histology change (Alzabin S and Williams RO, 2011; Rodeghero R et al., 2013). In our previous study, we have found that CK alleviated CIA and AA, downregulated the percentage of activated T-cells, and upregulated naive T-cells and Treg cells in spleen (Chen J et al., 2014; Liu KK et al., 2014). Consistent with these findings, the results in this study show that the severity of CIA was alleviated; naive T cells in the lymph nodes increased, whereas the activated T cells in the lymph nodes decreased when CIA mice were treated with CK. To further investigate the mechanism of CK responsible for suppressing T cell activation, we focused on DCs, the most powerful APCs in this study.

Second lymphoid organs, such as lymph nodes, are the main area where DCs interact with T cells and promote T cell activation. DC accumulation in the secondary lymphoid tissues is associated with inflammation. A greater number of DCs in the lymph nodes and increased Th2 inflammation have been found in hypersensitivity pneumonitis mice (Fanning LB et al., 2013). Our results show that CK downregulated the
percentage of DCs in the lymph nodes in CIA mice. Interestingly, percentage of DCs and naive T cells revealed a negative correlation, whereas percentage of DCs and the activated T cells revealed a positive correlation. These results suggest that the inhibitory effect of CK on T cell activation may be associated with decreasing DC accumulation in the lymph nodes.

Tissue inflammation induces rapid mobilization of antigen-charged DCs, which can migrate to the draining lymph nodes via afferent lymphatics to induce pathogenic T cells such as Th1 and Th17 (Ma N et al., 2013; Platt AM and Randolph GJ, 2013). Upregulation of CCR7 and CCL21 can lead to an increase of DCs in the lymph nodes, thus upregulating the development of inflammation. In mice model of hypersensitivity pneumonitis, the lung DCs of challenged Lilrb4(-/-) mice expressed more CCR7 and CCL21 and also contained significantly greater numbers of Ag-bearing DCs in the lymph nodes, and the attendant exacerbated the Th2 lung pathology (Fanning LB et al., 2013). Reducing the expression of CCR7 can inhibit DC migration to the lymph nodes and then limit the generation of adaptive immunity (Odobasic D et al., 2013). Our results show that CK decreased CCL21 level in the lymph nodes and CCR7 expression on DCs and suppressed CCL21-mediated migration of DCs. Interestingly, percentage of DCs and CCL21 level revealed a positive correlation ($R^2 = 0.7802$, $P = 0.0470$). These results suggest that
the effect of CK on reducing DC accumulation in the lymph nodes may be due to the decreasing expression of CCL21 and CCR7.

CCR7 facilitates the proinflammatory function of DCs in inflammation conditions. B6.CCR7(-/-) mice inoculated with B6.WT DCs(CCR7+) developed augmented leishmaniasis lesions, whereas WT mice injected with B6.CCR7(-/-) DCs presented delayed healing of the lesion (Kling JC et al., 2014). CCR7 and its ligands are associated with the severity of inflammation. In rheumatoid RA patients, the concentration of CCL19 and CCL21 in the plasma and synovial tissue, the cell-surface expression of CCR7 on circulating monocytes and CD4+ T cells, and the elevated level are associated with disease activity (Pickens SR et al., 2011; Ellingsen T et al., 2014). Inhibition of CCR7 in lesional skin is a critical event for clinical remission induced by TNF blockade in patients with psoriasis (Bosè F et al., 2013). Our results show that CK exerted therapeutic effect in CIA mice and decreased CCL21 and CCR7 expression. With our results and the studies previously mentioned as basis, we suppose that the suppression of CCL21 and CCR7 may be one of the mechanisms for the anti-inflammatory effect of CK.

T cell activation requires three major signals provided by APCs (Caporali R et al., 2014). Signal 1 consists of processed antigen peptide, which is presented in the context of MHC complexes to TCR. Signal 2 consists of upregulation of costimulatory molecules (such as CD80 and
CD86) for binding to their receptor (CD28) on T cells. Signal 3 consists of the proper cytokines, providing the final differentiation and proliferation of T cells. We observed that the ability of DCs to promote T cell proliferation was impaired by CK. In addition, this study showed that CK impaired the expression of CD80, CD86, and MHC II on DCs. Consistent with these results, our previous study showed that CK suppressed TCR and CD28 expression on T cells (Liu KK et al., 2014), reduced IL-2 production and CD25 (α chain of the high-affinity IL-2 receptor) expression on T cells (Chen J et al., 2014). These data suggest that CK impairs T cell activation by reducing the signals provided by DCs.

In our previous studies, CK increased the percentages of CD4⁺CD25⁺Foxp3⁺ Tregs in CIA mice (Liu KK et al., 2014). It is well established that Tregs play a critical protective role in RA and other autoimmune diseases. (Cooles FA et al., 2013). Tregs control the responses of APCs and effector T cells through direct interaction or through anti-inflammatory cytokine production, and they play an important role in limiting inflammation and regulating adaptive immunity (Li Q and Shen HH, 2009; Bacchetta R et al., 2007). Tregs co-localise with DC in the lymphoid aggregate areas of RA synovium, but cannot fully suppress DC’s activation and function (E XQ et al., 2012). In this study, we found CK suppressed DC priming T cell activation. Based on these studies, we
can suppose that the effect of CK on suppressing DC’s function may be associated with Tregs in CIA mice, but the exact effect and mechanism needed to be further studied.

Taken together, this study demonstrated that CK suppressed DC priming T cell activation in CIA, and suppressing CCL21/CCR7-mediated DC migration and signals between T cell and DC might be the potential mechanisms. These results provide an interesting novel insight into the potential mechanism by which CK contributes to the anti-inflammatory effect in autoimmune conditions. It's important to note that activation of T cells in vivo is complicated, the potential mechanisms of CK on T cells activation needed to be future studied.
Authorship Contributions

Participated in research design: Wei, Chen.

Conducted experiments: Chen, Wang.

Contributed new reagents or analytic tools: Wu, Chang, Liu.

Performed data analysis: Chen.

Wrote or contributed to the writing of the manuscript: Chen, Wei.
References


Caporali R, Bugatti S, Cavagna L, Antivalle M, and Sarzi-Puttini P (2014) Modulating the co-stimulatory signal for T cell activation in rheumatoid arthritis: could it be the first step of the treatment?


Kling JC, Darby J, and KÅ¶rner H (2014) CCR7 facilitates the


collagen-induced arthritis by regulating T and B lymphocytes function in DBA/1 mice. *Eur J Pharmacol* **654**:304-314


Footnotes

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Figure Legends

**Fig. 1 Chemical structure of CK (C_{36}H_{62}O_{8}, MW: 622.88) (20-O-D-glucopyranosyl-20(S)-protopanaxadiol)**

**Fig. 2 CK alleviated arthritis severity of CIA mice.** After onset of arthritis at d27, mice were given CK (once a day) and MTX (once 3 days) from day 27 to day 47. The arthritis global assessment and swollen joint count of mice were evaluated every 3 days. (A) arthritis global assessment, (B) swollen joint count. Values are expressed as a mean ± SD. n=10 for each group. *P < 0.05, **P < 0.01 vs CIA.

**Fig. 3 The effect of CK on subsets of T cells and DCs in lymph nodes from CIA mice.** Subsets of naïve T cells (A, D), activated T cells (B, E), and DCs (C, F) in CIA mice lymph nodes were evaluated by flow cytometry. The representative flow cytometry dot plot of each group is shown (A, B, C). A scatter plot of the correlation between DCs and naïve T cells (G), DCs and activated T cells (H). Values are expressed as a mean ± SD. n=6 for each group. ##P < 0.01 vs normal; *P < 0.05, **P < 0.01, ***P < 0.001 vs CIA.

**Fig. 4 CK suppressed DCs migration.** (A) Migration of DCs induced by CCL21 was evaluated by transwell assay. The migration index was calculated as the number of cells transmigrating with CCL21 divided by the number of transmigrating cells in the absence of CCL21. **P < 0.01 vs control. The results are representative of at least three independent
experiments. (B) CCL21 expression in CIA lymph nodes was assayed by ELISA. Data are expressed as mean ± SD, n=6 for each group. #P < 0.01 vs normal, *P < 0.05, **P < 0.01, vs CIA. (C) A scatter plot of the correlation between DCs and CXCL12 level in lymph nodes. (D, E) CCR7 expression on DCs was assayed by flow cytometry. Isotype (red line), control (blue line), CK 10μM (orange line). Mean fluorescence intensity is presented as mean ± SD. **P < 0.01 vs control. The results are representative of at least three independent experiments. (F, G) CCR7 expression on DCs was assayed by western blot. Data are expressed as mean ± SD, **P < 0.01, ***P < 0.001 vs control. The results are representative of at least three independent experiments.

**Fig. 5** CK suppressed T cell-stimulatory capability of DCs. T cell-stimulatory capability of DCs was assayed by mixed lymphocyte reaction. DCs and T cells (at a ratio of 1: 20) co-cultured for 44h, then proliferation of T cells was assayed by Cell Counting Kit assay. Data are expressed as mean ± SD. #P < 0.01 vs T cell; *P < 0.05, **P < 0.01 vs T cells with DCs. The results are representative of at least three independent experiments.

**Fig. 6** CK impaired the expression of CD80, CD86 and MHCII on DC. The expression of CD80 (A, B), CD86 (C, D) and MHCII (E, F) on DC were assayed by flow cytometry. Isotype (red line), control (blue line), CK10μM (orange line). Mean fluorescence intensity is presented as
mean ± SD. *P < 0.05, **P < 0.01 vs control. The results are representative of at least three independent experiments.
Fig. 1
Fig. 5

A450

- T
- DC+T
- (DC+CK 1 nM) + T
- (DC+CK 100 nM) + T
- (DC+CK 10 μM) + T

Bars are labeled with significance symbols: ##, *, **.
Figure A: Flow cytometry graph showing CD80 expression on DCs with different treatments.

Figure B: Bar graph showing CD80 expression mean fluorescence intensity.

Figure C: Flow cytometry graph showing CD86 expression on DCs with different treatments.

Figure D: Bar graph showing CD86 expression mean fluorescence intensity.

Figure E: Flow cytometry graph showing MHCII expression on DCs with different treatments.

Figure F: Bar graph showing MHCII expression mean fluorescence intensity.