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Research Paper

Anti-inflammatory effects of *Artemisia princeps* in antigen-stimulated T cells and regulatory T cells

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Abstract

Objectives The aim was to investigate the anti-inflammatory effects of *Artemisia princeps* extract on the activity of anti-CD3/CD28-stimulated $CD4^+CD25^-$ T cells and antigen-expanded regulatory T cells.
Methods $CD4^+CD25^-$ T cells were activated with coated anti-CD3 and anti-CD28 and cultured in the presence or absence of various concentrations of *A. princeps* extract. The cultures were pulsed on Day 6 with [^{3}H]thymidine and, after harvesting the cells, [^{3}H]thymidine incorporation was measured. For analysis of interleukin-2 and interferon-γ secreted from $CD4^+CD25^-$ T cells, culture supernatants were collected on Days 2 and 6. For the analysis of interleukin-10 secreted from the $CD4^+CD25^-$ T cells and expanded regulatory T cells, supernatants were collected after 2 and 7 days, respectively. Cytokine levels were determined using an enzyme-linked immunosorbent assay. Potential medicinal components of the *A. princeps* extract were determined using gas chromatography–mass spectrometry.
Key findings *A. princeps* (30 μg/ml) effectively suppressed proliferation of $CD4^+CD25^-$ T cells that were stimulated with anti-CD3/CD28 without causing cytotoxicity in spleen cells incubated under conditions lacking antigen stimulation. *A. princeps* inhibited production of the pro-inflammatory cytokines interleukin-2 and interferon-γ in anti-CD3/CD28-stimulated $CD4^+CD25^-$ T cells. Also, the extract slightly increased production of the anti-inflammatory cytokine interleukin-10 in these cells. In regulatory T cells expanded by anti-CD3/CD28, *A. princeps* increased production of interleukin-10 and Foxp3.
Conclusions The results suggest that *A. princeps* may be useful in the treatment of autoimmune diseases and organ transplantation rejection by inhibiting proliferation of inflammatory T cells, suppressing inflammatory processes in antigen-stimulated $CD4^+CD25^-$ T cells and increasing activity of expanded regulatory T cells.
Keywords anti-inflammatory; *Artemisia princeps*; cytokines; plant extract; T cells

Introduction

Artemisia princeps is one of the approximately 500 plants in the genus *Artemisia.* It has various uses, including as a medicine and food, in China, Korea and other Oriental countries. Several medicinal properties have been described. The plant has been reported to have an anti-inflammatory effect on lipopolysaccharide-treated macrophages[1–3] and it promotes endothelial cell division.[4] Moreover, it has been reported to induce anti-allergic,[5–7] antioxidant,[8,9] antidiabetic,[10,11] antitumour[12–14] and antibacterial effects.[15]

Despite this research, the anti-inflammatory effects of *A. princeps* on T cells, which play a crucial role in adaptive immunity, are largely unknown. More information is required on the mechanism by which the extracts modulate the activity of T cells.

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$CD4^+$ Th_1 and Th_2 cells are derived from a common pool of naive Th_0 cells.[16] The pro-inflammatory cytokines interleukin (IL)-2, interferon (IFN)-γ and tumour necrosis factor (TNF)-α were produced mainly by activated Th_1 cells,[17,18] and these proteins contribute to the pathogenesis of autoimmune diseases and allograft rejection. The synthesis of IL-2, IFN and granulocyte macrophage colony stimulating factor stimulate the production of cytokines such as TNF-α and, consequently, TNF-α and IFN induce IL-1 production.[19] In contrast, IL-10 produced by Th_2 cells inhibits Th_1 activity and strongly inhibits secretion of the pro-inflammatory cytokines IL-1 and TNF-α.[20] In-vitro, unprimed T cells of the individual T cells are activated and expanded in culture using anti-CD3 and anti-CD28 monoclonal antibodies.[21,22]

Regulatory T cells (Tregs) may inhibit autoreactive cells in autoimmune diseases such as colitis and gastritis.[23–26] Tregs have also been used to treat animal models of graft-versus-host disease.[27–29] The three different classes of Tregs are naturally occurring $CD4^+CD25^+$ T cells (nTregs), IL-10-producing Tr1 cells and transforming growth factor (TGF)-β-producing Th3 cells.[30] Also, nTregs were called naïve $CD4^+CD25^+$ T cells. nTregs, which express the transcription factor Foxp3 and secrete cytokines such as IL-10 and TGF-β, constitute approximately 10% of peripheral $CD4^+$ T cells in mice and humans.[23,31,32]

Allograft rejection and autoimmunity is contributed to mainly by both $CD4^+$ and $CD8^+$ T cell-mediated immune responses.[33–37] Thus, regulation of the action of $CD4^+$ and $CD8^+$ T cells is important in the treatment of these diseases. Also, the development of novel immunotherapeutic agents with reduced side-effects is required.

In this study, we investigated whether *A. princeps* can inhibit the proliferation of anti-CD3/CD28-stimulated $CD4^+CD25^-$ T cells *in vitro*, which were obtained after naïve $CD4^+CD25^+$ T cells were removed from the pool of $CD4^+$ T cells. Additionally, we investigated whether the extract decreases the production of the pro-inflammatory cytokines IL-2 and IFN-γ or increases the production of the anti-inflammatory cytokine IL-10 in antigen-stimulated $CD4^+CD25^-$ T cells. Finally, this study addressed the influence of *A. princeps* on the activity of expanded Tregs.

Materials and Methods

Animals

Male C57BL/6 mice (aged 8–12 weeks) were purchased from Orient Bio Inc. (Seongnam, Korea) and kept under specified pathogen-free conditions in accordance with protocols for animal experiments approved by the Animal Use and Care Committee of the Ulsan University (no. 2007-13-088).

Preparation of a crude extract of *A. princeps*

In order to prepare the extract, the sample from the trunk of *A. princeps* (30–40 g) was treated with 200 ml of methanol at 50°C, 1500 psi for 3 days by using an ultrasonic cleaner (Branson Ultrasonics Corp., CT, USA); the extracted materials were concentrated with a speed bag (Modul spin 40; Biotron Corporation, Gangwondo, Korea) at 40°C for 24 h. Thereafter, the *A. princeps* methanol extract was dissolved to attain a concentration of 20 mg/ml in phosphate-buffered saline (PBS), and diluted to 3–30 μg/ml for the in-vitro assay using the spleen, $CD4^+CD25^-$ T cells and Tregs.

Cell purification and culture

$CD4^+$ T cells were enriched from spleen and lymph node cells by negative selection using a $CD4^+$ T cells isolation kit (Miltenyi Biotec, CA, USA) according to the manufacturer's instructions. The isolated $CD4^+$ T cells were incubated for 15 min at 4°C with fluorescein isothiocyanate (FITC)-conjugated anti-CD4 antibody (Ab) and phycoerythrin-conjugated anti-CD25 Ab in PBS, and the $CD4^+$ $CD25^-$ T and $CD4^+$ $CD25^+$ T cell populations were isolated using a FACSVantage cell sorter (BD Biosciences, CA, USA). The isolated $CD4^+CD25^-$ T cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine and 55 μM 2-mercaptoethanol at 37°C under 5% CO_2 in humidified air (all reagents were purchased from Life Technologies, NY, USA).

Cell proliferation analysis

$CD4^+CD25^-$ T cells (3×10^4 cells per well in 96-well U-bottom plates) were activated with 5 μg/ml coated anti-CD3 (145-2C11; BD Biosciences) and anti-CD28 (37.51; BD Biosciences), respectively, and cultured for 7 days in the presence or absence of various concentrations of *A. princeps* extract, which was added to the medium on alternate days. The cultures were pulsed on Day 6 with 1 μCi [^{3}H]thymidine per well for the last 18 h. After harvesting the cells, [^{3}H] thymidine incorporation was measured as counts/min using a β-plate counter.[22]

Cell viability analysis

Cell viability was determined using a Cell Counting Kit (CCK)-8 (Dojindo Laboratories, Kumamoto, Japan), according to the manufacturer's instructions. In brief, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (WST-8) is reduced by dehydrogenases in cells yielding an orange-coloured product (formazan).[38] The amount of formazan dye generated by the dehydrogenases in the cells is directly proportional to the number of living cells. Spleen cells (3×10^4 cells/well in 200 μl/well complete RPMI-1640 media in a 96-well U-bottom plate) were cultured without antigen stimulation in the presence or absence of 30 μg/ml *A. princeps* extract for 3 days. CCK-8 (20 μl) was added to each well, and the cells were incubated for 2–3 h. The absorbance was measured at 450 nm using a microplate reader.

Cytokine analysis

For analysis of IL-2 and IFN-γ secreted from $CD4^+CD25^-$ T cells (3×10^4 cells/well in 96-well U-bottom plates), the culture supernatants were collected on Days 2 and 6. For the analysis of IL-10 secreted from the $CD4^+CD25^-$ T cells and the expanded Tregs (1.5×10^5 cells per well in 6-well plates), the supernatants were collected after 2 and 7 days, respectively. Cytokine levels were determined using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, MN, USA).

Expansion of $CD4^+CD25^+$ T cells

Isolated naïve $CD4^+CD25^+$ T cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine and 55 μM 2-mercaptoethanol containing 1000 U/ml rhIL-2 (Proleukine; Chiron, Amsterdam, The Netherlands) at 37°C, 5% CO_2 in humidified air. In addition, the isolated cells were expanded with 5 μg/ml coated anti-CD3 and anti-CD28, respectively. Expanded Tregs were washed after 1 week in culture, and used to investigate the effect of *A. princeps* extract on the production of Foxp3 and IL-10.

Flow cytometric analysis of Foxp3

Expanded Tregs (1.5×10^5 cells/well in 6-well plates) were incubated in the presence or absence of *A. princeps* extract for 7 days. The cells were washed in PBS and incubated in a fixation/permeabilization solution for 2–3 h. For analysis of Foxp3, the cells were stained using an APC anti-mouse Foxp3 staining set, according to the manufacturer's instructions (eBioscience). The cells were stained with 0.5 μg/ml of APC anti-mouse Foxp3 Ab. APC isotype-matched Ab (0.5 μg/ml) (rat IgG2a; eBioscience, CA, USA) was used for staining the control for Foxp3. Flow cytometric analysis was performed on a FACSCalibur flow cytometer (BD Bioscience).

Gas chromatography-mass spectrometry analysis

Gas chromatography–mass spectrometry (GC-MS) analysis of the *A. princeps* methanol extract was performed on an Agilent 6890 GC/5973N mass selective detector (Agilent Technologies, Palo Alto, CA, USA); a high resolution capillary column DM-5MS (30 m $\times$ 250 μm $\times$ 0.25 μm) was used. The oven temperature was initially set at 60°C for 3 min, then increased to 310°C at a rate of 5°C/min and was maintained for 17 min. The front inlet temperature was maintained at 250°C. Split injection was conducted with a split ratio of 10 : 1, and helium was used as the carrier gas at a flow rate of 1 ml/min; 1 μl of the sample was injected for analysis.

Statistical analysis

Statistically significant differences among the multiple groups were tested with the Kruskal–Wallis test with Dunn's post-hoc test to identify individual groups versus control. To detect significant differences between the two groups the Mann–Whitney test was used. All results were generated using GraphPad Prism software (CA, USA).

Results

Effects of *A. princeps* on proliferation of antigen-stimulated $CD4^+CD25^-$ T cells

$CD4^+$ T cells were enriched from spleen and lymph node cells using negative selection, and $CD4^+CD25^-$ T cells and $CD4^+CD25^+$ T cells were isolated from $CD4^+$ T cells using a FACSVantage cell sorter. The purity of $CD4^+CD25^-$ T cells and $CD4^+CD25^+$ T cells was 97–99% and 96–98%, respectively (Figure 1). Anti-inflammatory effects of *A. princeps* have been reported in macrophages and mast cells. However, whether extracts have the anti-inflammatory action in T cells is not known. We therefore investigated whether *A. princeps* extract inhibited antigen-stimulated T cell proliferation.

A. princeps (30 μg/ml) effectively inhibited anti-CD3/CD28-stimulated $CD4^+CD25^-$ T cell proliferation ($P < 0.001$) (Table 1). However, the extract was not cytotoxic to spleen cells that lacked antigen stimulation for 3 days (Figure 2a).

To confirm these results, we determined the time course of this inhibition by incubating *A. princeps* extract (30 μg/ml) in anti-CD3/CD28-stimulated $CD4^+CD25^-$ T cells for 3–7 days. The extract suppressed proliferation of the antigen-stimulated $CD4^+CD25^-$ T cells. This occurred on Day 7 when the control showed a peak of 42% inhibition of proliferation ($P = 0.0006$) (Figure 2b). These results showed that the inhibitory effects of the extract were dose and time dependent.

Effects of *A. princeps* on production of IL-2 and IFN-γ in antigen-stimulated $CD4^+CD25^-$ T cells

We investigated the effects of *A. princeps* (30 μg/ml) on the production of the pro-inflammatory proteins IL-2 and IFN-γ, which are the major cytokines secreted by Th_1 cells. $CD4^+CD25^-$ T cells were activated with anti-CD3/CD28 Ab in the presence or absence of the *A. princeps* extract, and IL-2 and IFN-γ secretion was analysed on Days 2 and 6 using ELISA.

On Day 6, $CD4^+CD25^-$ T cells produced less IL-2 ($P = 0.0414$) and IFN-γ ($P = 0.0009$) in the presence of the *A. princeps* extract compared with that secreted by cells cultured without the plant extract (Table 2). This suggests that the extract can decrease the production of pro-inflammatory cytokines in antigen-stimulated $CD4^+CD25^-$ T cells.

Effects of *A. princeps* on production of IL-10 in antigen-stimulated $CD4^+CD25^-$ T cells and expanded regulatory T cells

$CD4^+CD25^-$ T cells were activated with anti-CD3/CD28 Ab in the presence or absence of the *A. princeps* extract (30 μg/ml) to investigate the effects of the plant on production of the anti-inflammatory cytokine IL-10. Secretion of IL-10 was analysed on Day 2 using ELISA. On Day 2, the cells grown in the presence of the extract produced slightly more IL-10 ($P = 0.0715$) than the control (Table 3), indicating that the extract caused partial differentiation of Th_0 into Th_2 cells.[18]

We also investigated whether *A. princeps* influences the production of IL-10 in expanded Tregs. First, nTregs were expanded by anti-CD3/CD28 Ab, which resulted in up to 41-fold expansion within 21 days (Figure 3a). Next, we used the Tregs that were expanded over 7 days to determine the influence of the extract on production of IL-10. The expanded Tregs were incubated with or without *A. princeps* (30 μg/ml) for an additional 7 days, adding the extract every other day. The extract increased production of IL-10 up to 1.37-fold over that seen in the control at 7 days ($P = 0.0426$) (Table 3).

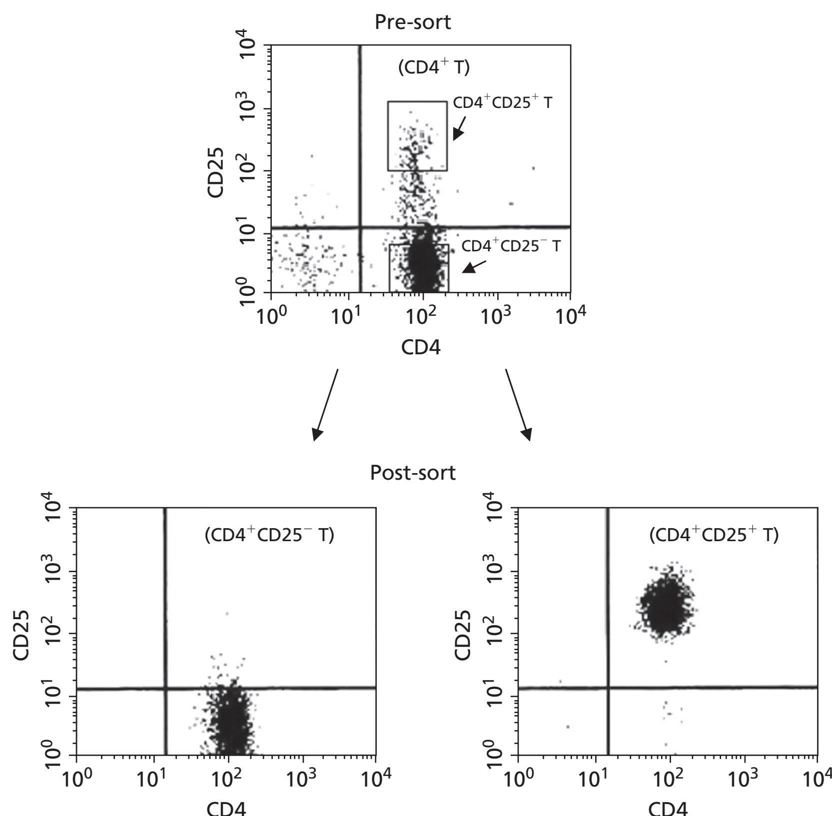

Figure 1 Purification of CD4$^+$CD25$^-$ T and CD4$^+$CD25$^+$ T cells from spleen. CD4$^+$ T cells isolated from spleen cells were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-CD4 and phycoerythrin (PE)-conjugated anti-CD25 antibody for 15 min at 4°C and sorted in a FACSVantage cells sorter. The isolated CD4$^+$CD25$^-$ T cells and CD4$^+$CD25$^+$ T cells were 97–99% and 96–98% pure, respectively. Dot plots show CD4-FITC on the *x*-axis and CD25-PE on the *y*-axis.

Effects of *A. princeps* on expression of Foxp3 in expanded regulatory T cells

We investigated whether *A. princeps* influences expression of Foxp3 in expanded Tregs. To test this, the nTregs were expanded using anti-CD3/CD28 over 7 days. The expanded Tregs were cultured with *A. princeps* (30 μg/ml) for an additional 7 days, adding the extract every other day. The results showed that the extract increased expression of Foxp3 up to 5.36% in contrast with the control at 7 days (Figure 3b).

Table 1 Inhibitory effect of *Artemisia princeps* on the proliferation of antigen-stimulated CD4$^+$CD25$^-$ T cells

Treatment	Concentration (μg/ml)	Proliferation (counts/min)
		CD4$^+$CD25$^-$ T cells
Control	0	8454 ± 416
A. princeps	3	7455 ± 929.5
	10	6459 ± 710.3*
	30	4911 ± 725.1***

CD4$^+$CD25$^-$ T cells were activated with 5 μg/ml anti-CD3 and anti-CD28, respectively, and incubated in the presence or absence of various concentrations of *A. princeps* for 7 days, which was added on alternate days. On Day 6, the cultures were pulsed with 1 μCi [^{3}H]thymidine per well for the last 18 h and the cells were harvested and counted. Data are presented as mean ± SEM from four independent experiments. *$P < 0.05$, ***$P < 0.001$, significantly different compared with the control.

Volatile components of *A. princeps* methanol extract identified by GC-MS

We identified the potential medicinal components of the *A. princeps* extract using GC-MS. Compounds were identified by comparison with those in the library (in the library search program hits that were >90% probable were viewed as likely hits). Table 4 lists the chemical components of the extract. The analysis yielded 15 compounds with anti-inflammatory properties: borneol L, spathulenol, neophytadiene, phytol, scopoletin, *trans*-phytol, 9,12-octadecadienoic acid (*Z*,*Z*)-, stearic acid, matricarin, linolein, 1-mono-, vitamin E, campesterol, stigmasterol, γ-sitosterol and α-amyrin.

Discussion

This study was an immunological investigation of a crude *A. princeps* extract in order to assess its potential as a treatment for autoimmune diseases and organ transplant rejection. The anti-inflammatory properties of the extract were determined in antigen-stimulated CD4$^+$CD25$^-$ T cells and Tregs expanded with an antigen. We specifically

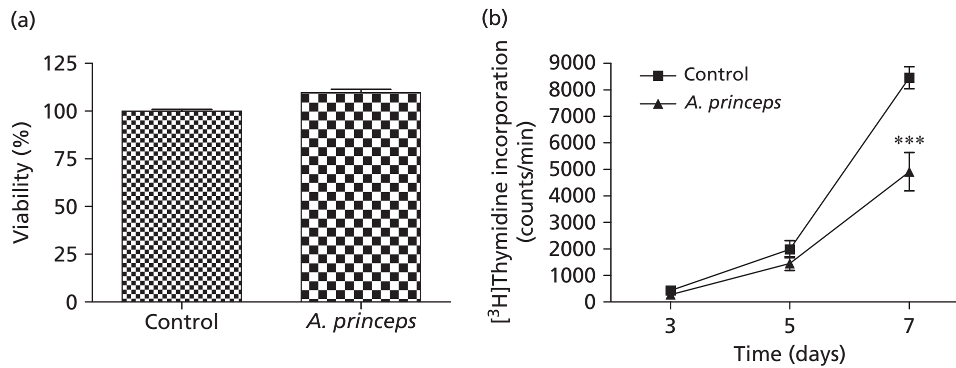

Figure 2 Cell viability in spleen cells and curve plots of $CD4^+CD25^-$ T cell proliferation over time. (a) Cell viability was determined using the CCK-8 assay. Spleen cells were incubated with 30 μg/ml *Artemisia princeps* in the absence of anti-CD3/CD28 for 3 days. (b) Anti-CD3/CD28-mediated $CD4^+CD25^-$ T cells were incubated on 96-well plates in the presence or absence of 30 μg/ml *A. princeps*. Cell proliferation was determined over 3–7 days. The cultures were pulsed with 1 μCi [^{3}H]thymidine per well for the last 18 h, and the cells were harvested and counted. Data are presented as the mean ± SEM from three independent experiments. ***$P < 0.001$, significantly different compared with the control.

examined the following: whether an *A. princeps* extract inhibits proliferation of $CD4^+CD25^-$ T cells that were stimulated using anti-CD3/CD28; whether this extract inhibits production of the pro-inflammatory cytokines IL-2 and IFN-γ, and increases production of the anti-inflammatory cytokine IL-10 in anti-CD3/CD28-stimulated $CD4^+CD25^-$ T cells; and whether the extract influences the production of the transcription factor Foxp3 and IL-10 in expanded Tregs.[20,39,40]

Activated inflammatory T cells are involved in pathological processes such as autoimmunity and graft rejection.[41,42] The activated cells stimulate the release of pro-inflammatory cytokines from innate immune cells. The pathogenesis of spontaneous autoimmune diabetes in inbred non-obese diabetic mice was shown to be caused by the activity of $CD4^+$ and $CD8^+$ T cells.[43] This confirms the importance of controlling inflammatory T cells for the successful treatment of diseases of these types. We showed in this study that *A. princeps* (30 μg/ml) effectively suppressed proliferation of anti-CD3/CD28-stimulated $CD4^+CD25^-$ T cells ($P < 0.001$; Table 1). A time course analysis was used to confirm this finding. To this end, the antigen-stimulated $CD4^+CD25^-$ T cells were incubated in the presence or absence of the *A. princeps* extract for 3–7 days (Figure 2b). The extract markedly suppressed antigen-stimulated $CD4^+CD25^-$ T cells proliferation on Day 7, whereas peak inhibition of proliferation was 42% for the control. This shows that the extract suppressed adaptive immunity. $CD4^+$ Th_1 cells mainly produce the pro-inflammatory cytokines IL-2 and IFN-γ. Table 2 showed that *A. princeps* decreased production of both IL-2 and IFN-γ in anti-CD3/CD28-stimulated $CD4^+CD25^-$ T cells at 6 days. In addition, the extract slightly increased production of IL-10 in anti-CD3/CD28-stimulated $CD4^+CD25^-$ T cells. This suggests that *A. princeps* may influence partial differentiation of Th_0 into Th_2 cells.[18,44]

Tregs are reported to be important regulators of autoimmunity[24,45,46] and transplantation tolerance.[47–50] This has spurred research on the expansion of Tregs. Earle *et al*,[40] for example, reported that Tregs cultured with anti-CD3/CD28 beads for 4 weeks expanded 200-fold. In this study, we expanded Tregs using anti-CD3/CD28, which caused a 41-fold expansion within 21 days (Figure 3a). We also investigated whether the extract increased the function of expanded Tregs. The *A. princeps* extract did indeed increase production of IL-10 (Table 3) and expression of Foxp3 (Figure 3b) in expanded Tregs.

Based on the results of this study, we suggest that *A. princeps* has anti-inflammatory activity with several underlying mechanisms: inhibition of $CD4^+CD25^-$ T cell proliferation following antigen-stimulation; decreased production of IL-2 and IFN-γ, and increased production of IL-10 in antigen-stimulated $CD4^+CD25^-$ T cells; and increased expression of Foxp3 and production of IL-10 in Tregs expanded using anti-CD3/CD28. Thus, *A. princeps* extract may be useful in the treatment of inflammatory pathologies

Table 2 Inhibitory effects of *Artemisia princeps* on the production of interleukin-2 and interferon-γ from antigen-stimulated $CD4^+CD25^-$ T cells

Treatment	**Interleukin-2 (pg/ml)**		**Interferon-γ (pg/ml)**	
	2 days	6 days	2 days	6 days
Control	38.41 ± 10.11	205.3 ± 22.78	41.69 ± 10.6	94.54 ± 12.23
A. princeps	14.03 ± 3.997	111 ± 20.67*	31 ± 7.815	44.27 ± 5.699***

Anti-CD3/CD28-stimulated $CD4^+CD25^-$ T cells were incubated with or without 30 μg/ml *A. princeps*, which was added on alternate days. The culture supernatant was collected at 2 and 6 days. Data are presented as mean ± SEM from three independent experiments. *$P < 0.05$, ***$P < 0.001$, significantly different compared with the control.

Table 3 Effects of *Artemisia princeps* on the production of interleukin-10 from antigen-stimulated CD4$^+$CD25$^-$ T and expanded regulatory T cells

Treatment	Interleukin-10 (pg/ml)	
	CD4$^+$CD25$^-$ T cells	Expanded regulatory T cells
Control	19.85 ± 5.196	929.8 ± 0.1949
A. princeps	57.3 ± 18.32	1275 ± 133.1*

Anti-CD3/CD28-stimulated CD4$^+$CD25$^-$ T cells were incubated with or without 30 μg/ml *A. princeps*. The culture supernatant of CD4$^+$CD25$^-$ T and expanded regulatory T cells was collected at 2 and 7 days, respectively. Data are presented as mean ± SEM from three independent experiments. *$P < 0.05$, significantly different compared with the control.

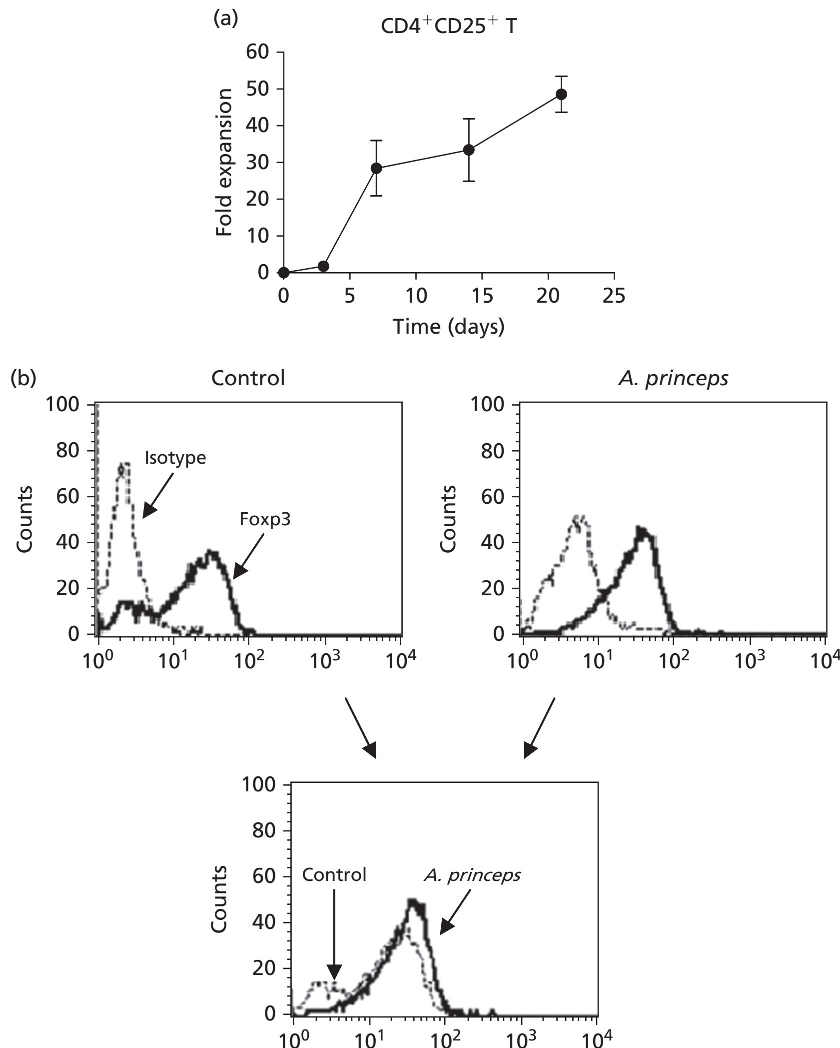

Figure 3 Effects of *Artemisia princeps* on expression of Foxp3 in expanded Tregs. (a) CD4$^+$CD25$^+$ T cells (nTregs) isolated from CD4$^+$ T cells were expanded in 5 μg/ml coated anti-CD3 and anti-CD28, respectively, for 0–21 days. The cells were incubated in new plates coated with anti-CD3/CD28 every week. (b) Expanded Tregs were incubated in the presence or absence of the *A. princeps* extract for 7 days. The cells were stained with 0.5 μg/ml of APC anti-mouse Foxp3 antibody. The staining control for Foxp3 was 0.5 μg/ml of APC isotype-matched antibody (rat IgG2a). Flow cytometric analysis was performed on a FACSCalibur flow cytometer. Data represent the findings of four independent experiments.

including autoimmune disorders and graft rejection through both anti-inflammatory activity of antigen-stimulated CD4$^+$CD25$^-$ T cells and increasing the activity of expanded Tregs.

The volatile components of the *A. princeps* methanol extract were identified using GC-MS. Fifteen of these compounds have anti-inflammatory properties (Table 4). Further study is necessary to determine the role of each of the

Table 4 Volatile compounds identified in *Artemisia princeps*

Retention time (min)	Compounds	Quality (%)	Peak area (arbitrary units)
1.97	Acetic acid	90	5480647
9.10	1-Methyl-4-(1-methylethyl)-benzene	93	480970
12.44	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	91	3252387
12.65	Camphor	96	349903
13.35	Borneol L	90	845981
14.98	Methylethylmaleimide	90	323934
22.74	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	96	859293
23.94	(+) Spathulenol	93	1985908
29.59	Neophytadiene	99	12306258
30.46	Phytol	90	3608417
31.83	Scopoletin	94	2309469
32.10	Palmitic acid	99	35337797
34.87	*trans*-Phytol	95	25011546
35.32	9,12-Octadecadienoic acid (Z,Z)-	99	18884505
35.43	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	94	32504701
35.82	Stearic acid	99	2667708
36.19	Butyl citrate	90	12061372
39.49	Matricarin	99	3358644
41.59	Palmitic acid β-monoglyceride	91	8696538
44.25	Linolein, 1-mono-	90	3593797
44.35	Octadeca-9,12,15-trien-1-ol	90	4737950
50.14	Vitamin E	95	1796941
51.32	Campesterol	99	3467885
51.64	Stigmasterol	95	17025567
52.40	γ-Sitosterol	99	23168237
53.47	α-Amyrin	95	9555633

15 compounds in the anti-inflammatory effects of the extract in activated T cells and Tregs.

Conclusions

This is the first study to show that an *A. princeps* methanol extract has anti-inflammatory effects in anti-CD3/CD28-stimulated $CD4^+CD25^-$ T cells and expanded Tregs. We showed that *A. princeps* decreased production of the pro-inflammatory cytokines IL-2 and IFN-γ in $CD4^+CD25^-$ T cells that were stimulated with an anti-CD3/CD28 Ab. The extract also slightly increased production of the anti-inflammatory cytokine IL-10 in these cells, which suggests a role in partial differentiation of Th_0 into Th_2 cells. *A. princeps* increased production of IL-10 and Foxp3, demonstrating its anti-inflammatory effect in expanded Tregs. Our studies suggest that *A. princeps* may be useful in the treatment of inflammatory pathologies involving autoimmune diseases and transplantation tolerance. Our understanding of the mechanism underlying the anti-inflammatory activity of the extract in activated T cells and expanded Tregs will improve with further study, and the active components of *A. princeps* remain to be identified.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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