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Effect of astragaloside Ⅳ on T, B lymphocyte proliferation and peritoneal macrophage function in mice[1]

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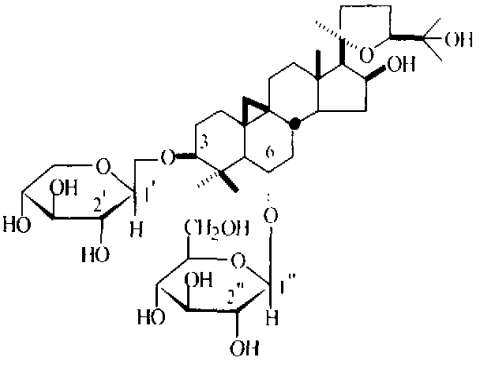
KEY WORDS *Astragalus membranaceus*; saponins; T-lymphocytes; B-lymphocytes; antibody formation; peritoneal macrophages; interleukin-1; tumor necrosis factor

ABSTRACT

AIM: To investigate the effect of astragaloside Ⅳ (ASI) on T, B lymphocyte proliferation, antibody production, and cytokines produced by murine peritoneal macrophages. **METHODS**: MTT assay was used to determine T, B lymphocyte proliferation and quantitative hemolysin spectrophotometry (QHS) assay was applied to test antibody production; IL-1 production was measured by thymocyte proliferation assay; TNF-α production was determined by the cytotoxicity assay against L929 cells. **RESULTS**: 1) *In vivo*, ASI 50-200 mg/kg ig for 7 d increased T lymphocyte proliferation and antibody production, and ASI 50-100 mg/kg ig for 7 d increased B lymphocyte proliferation but ASI 200 mg/kg had no effect on B lymphocyte proliferation; 2) *In vitro*, ASI increased T, B lymphocyte proliferation only at 100 nmol/L; 3) ASI increased IL-1 activity at 1 nmol/L *in vitro*, but decreased it at 100 and 1000 nmol/L; 4) ASI inhibited TNF-α activity with or without LPS-stimulation *in vitro*. **CONCLUSION**: ASI increased T, B lymphocyte proliferation and antibody production *in vivo* and *in vitro*; but inhibited productions of IL-1 and TNF-α from peritoneal macrophages *in vitro*.

INTRODUCTION

Astragalus membranaceus (Fisch) Bge (*Am*) is a traditional Chinese medicine, used as a tonic herb in many immuno-deficient diseases[1]. Its chemical components including polysaccharides, flavonoids, and saponins. The immunomodulatory effects of *Am* crude extracts and its components were reported[2]. In our previous study, the fraction of saponin was found to be active, which had anti-inflammatory and immunomodulatory effects. Recently it is reported that astragalus saponins were used in treating rheumatoid arthritis (RA)[3]. Astragaloside Ⅳ (ASI) is a pure saponin isolated from radix of *Am*, which is a diglycoside of cycloastragens possessing one glucoside moiety and one xyloside moiety[4]. In this experiment, the effects of ASI on T, B lymphocyte proliferation and antibody production *in vivo*, and the effect of ASI on IL-1 and TNF-α from peritoneal macrophages *in vitro* were investigated.



Astragaloside Ⅳ

MATERIALS AND METHODS

Astragalosides Astragalosides (AST) is the total saponin fraction isolated from Shanxi *Astragalus membranaceus* (Fisch) Bge by MeOH extract and n-BuOH distribution. AST is a white powder, ground, and suspended in 0.1 % CMC.

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ASI ASI is colorless needles crystalized from AST by MeOH, mp 299 – 301 ℃, purity > 95 %. ASI was ground and suspended in 0.1 % CMC when given *in vivo*, dissolved by 70 % ethanol (< 0.02 %) and then diluted when studied *in vitro*.

Mice Inbred ICR mice (6 – 8 weeks old, 18 – 22 g, female); BALB/c mice (6 – 8 weeks old, 20 g ± 2 g, female, for IL-1 assay) were purchased from Shanghai Experimental Animal Center, Chinese Academy of Sciences (Grade Ⅱ, Certificate № 153).

Cell line L929 cell (tumorigenic murine fibroblast) was kindly supplied by Ms XIN Shun-Mei (Shanghai Institute of Cell Biology, Chinese Academy of Sciences).

Reagents Concanavalin (ConA, № 27700) and lipopolysaccharides (LPS, № 62326) from *E coli* were purchased from Sigma Co. Actinomycin D and 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Fluka Co. Medium RPMI-1640 was purchased from Gibco Co. All RPMI-1640 media were supplemented with HEPES buffer 10 mmol/L, benzylpenicillin 100 kU/L, streptomycin 100 mg/L, *L*-glutamine 2 mmol/L, 2-mercaptoethanol 50 μmol/L, and 10 % fetal bovine serum, pH 7.2. Crystal violet was supplied by Shanghai Chongming Chemical Reagent Factory.

Lymphocyte proliferation assay[5] Mouse spleen was torn and passed through a stainless mesh (100 Φ). The single cell suspension was adjusted to 5×10^9/L. T or B cell proliferation was stimulated by ConA (final concentration 5 mg/L) and LPS (final concentration 10 mg/L) respectively. After incubated at 37 ℃, 5 % CO_2 for 44 h, MTT (5 g/L) 20 μL was added for another 4 h and then the solution (10 % SDS, 50 % dimethyl formamide, pH 7.4) 100 μL was added into each well. The absorbance was measured at 570 nm by microplate spectrophotometer (Bio-RAD, Model 550, Microplate Reader).

Antibody activity assay[6] Antibody activity from lymphocyte was measured by quantitative hemolysin spectrophotometry (QHS) assay. The mice was challenged by ip 5 % sheep red blood cell (SRBC) 0.2 mL per mouse on d 3. ASI and AST were given to the mice by ig at 50, 100, and 200 mg/kg for 7 d. The splenocyte suspension was adjusted to 1×10^{10}/L, incubated with sheep erythrocyte and guinea pig serum 1 mL respectively at 37 ℃ for 1.5 h, then centrifuged at 500 × *g* for 15 min. The supernatant was measured at 520 nm by UV-754 spectrophotometer as antibody activity.

IL-1 assay[7] IL-1 production was measured by thymocyte proliferation assay. Suspension of thymocyte 100 μL (2×10^{10}/L) taken from BALB/c mice was co-incubated with the supernatant of peritoneal macrophages 100 μL in the presence of ConA (5 mg/L) in a flat-bottomed 96-well microtiter plate for 72 h in a 37 ℃ 5 % CO_2 incubator. MTT 20 μL was added and incubated for 4 h. Absorbance was determined at 570 nm by microplate spectrophotometer.

TNF-α assay[8] TNF-α activity was determined by the cytotoxicity assay against L929 cells as described previously. L929 cells (4×10^8/L) were incubated for 24 h to form a single-layered cell, then medium was depleted and supernatant of TNF-α containing actinomycin D was added (final concentration of 2 mg/L). The plate was incubated for 20 h in a 37 ℃ 5 % CO_2 incubator. The cells were stained by 0.5 % crystal violet for 15 min. Plate was rinsed and dried. The residue was dissolved by 10 % SDS and absorbance was measured at 570 nm by microplate spectrophotometer. TNF-α activity was calculated as following:

$$\% \text{ Cytotoxicity} = (A_{control} - A_{test})/A_{control} \times 100\ \%$$

Statistics The data were expressed as $x \pm s$ and analyzed by t test.

RESULTS

Effects of AST and ASI on T, B lymphocyte proliferation and antibody production *in vivo* AST and ASI both affected T lymphocyte proliferation and antibody production at the dose of 50 – 200 mg/kg when given to ICR mouse by ig daily for 7 d. The effects of ASI on promoting immunological responses were stronger than that of AST. ASI 50 – 100 mg/kg increased B lymphocyte proliferation. But AST and ASI 200 mg/kg had no effects on B lymphocyte proliferation (Fig 1).

Effects of ASI on T, B lymphocyte proliferation *in vitro* ASI at 100 nmol/L promoted ConA-induced T lymphocyte proliferation and LPS-induced B lymphocyte proliferation ($P < 0.05$) but has little effect on resting lymphocyte proliferation ($P > 0.05$) (Tab 1).

Effect of ASI on IL-1 production from peritoneal macrophages *in vitro* ASI at 1 nmol/L markedly enhanced IL-1 activity with or without LPS stimulation but exhibited inhibitory effect at 100, 1000 nmol/L (Tab 2).

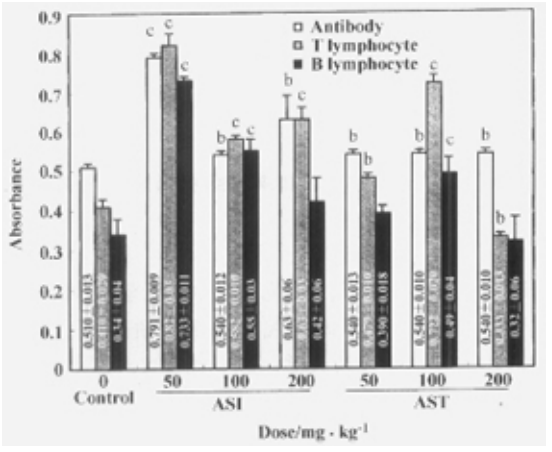

Fig 1. Effect of AST and ASI ig for 7 d on T, B lymphocyte proliferation and antibody production in mice. $n = 6$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control. Absorbance for antibody: $A_{520\ nm}$; for T, B lymphocyte: $A_{570\ nm}$.

Tab 1. Effect of ASI on T, B lymphocyte proliferation *in vitro*. $n = 3$. $\bar{x} \pm s$. $^bP < 0.05$ *vs* control group.

Concentration/ nmol·L^{-1}	$A_{570\ nm}$		
	Resting	ConA	LPS
0 (Control)	0.15 ± 0.03	0.337 ± 0.011	0.58 ± 0.03
0.1	0.133 ± 0.015	0.347 ± 0.021	0.59 ± 0.03
1	0.160 ± 0.010	0.38 ± 0.04	0.59 ± 0.03
10	0.160 ± 0.010	0.35 ± 0.04	0.65 ± 0.06
100	0.147 ± 0.015	0.367 ± 0.006^b	0.647 ± 0.011^b
1000	0.16 ± 0.03	0.347 ± 0.012	0.63 ± 0.05

Tab 2. Effect of ASI on IL-1 production from peritoneal macrophages *in vitro*. $n = 3$. $\bar{x} \pm s$. $^bP < 0.05$ *vs* control.

Concentration/ nmol·L^{-1}	$A_{570\ nm}$	
	Without LPS	With LPS
0 (Control)	0.49 ± 0.03	0.548 ± 0.010
0.1	0.48 ± 0.07	0.65 ± 0.09
1	0.78 ± 0.10^b	0.75 ± 0.06^b
10	0.493 ± 0.020	0.51 ± 0.00
100	0.393 ± 0.010^b	0.503 ± 0.010^b
1000	0.393 ± 0.010^b	0.50 ± 0.00^b

Effect of ASI on activity of TNF-α from peritoneal macrophages *in vitro* ASI inhibited TNF-α activity at 100 and 1000 nmol/L without LPS-stimulation and at 10 – 1000 nmol/L with LPS-stimulation (Tab 3).

Tab 3. Effect of ASI on activity of TNF-α produced from macrophages *in vitro*. $n = 3$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Concentration/ nmol·L^{-1}	Cytotoxicity/%	
	Without LPS	With LPS
0 (Control)	10.2 ± 0.8	34.7 ± 1.4
0.1	11.6 ± 2.1	30 ± 5
1	14 ± 5	31 ± 5
10	8.3 ± 2.4	30.6 ± 1.4^b
100	6.9 ± 1.4^b	29 ± 3^b
1000	1.4 ± 1.4^c	26.9 ± 0.8^c

DISCUSSION

Am is a traditional herb used in China with long history, the components of *Am* have been reported to enhance immune function in mice[9]. Saponins are some active components of *Am*. It has been reported that saponins have immunomodulatory and anti-inflammatory effects, used in the treatment for rheumatoid arthritis[10]. ASI is the main purified component of saponins.

In this study, ASI (50 – 200 mg/kg) enhanced T, B lymphocyte proliferation and antibody production in mice *in vivo*, similar with the effects of AST, indicating that ASI was an effective component of AST.

As we know, macrophages secrete pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α, *etc*. Once they are activated *in vivo*, the effects are magnified by LPS stimulation *in vitro*[11]. IL-1 and TNF-α are important cytokines with diverse biological functions, and play key roles in immune responses. *In vitro* studies, ASI (100 – 1000 nmol/L) suppressed both IL-1 and TNF-α production in the absence and presence of LPS from peritoneal macrophages. It suggested that ASI inhibited secretion of IL-1 and TNF-α from peritoneal macrophages directly at high concentration. The effects of ASI on macrophages may provide an explanation for the mechanism of its anti-inflammatory action *in vivo*[12], suggesting the possible application of this novel agent in the treatment of some inflammatory disorders, such as rheumatoid arthritis. But it was worthy to point out that ASI enhanced IL-1 production at low concentration (1 nmol/L) *in vitro*. The different role of ASI on IL-1 production showed immunomodulating action of ASI.

In conclusion, ASI enhanced T, B lymphocyte

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proliferation and antibody production, but inhibited IL-1 and TNF-α production from peritoneal macrophages at high concentration *in vitro*, indicating that ASI is an potent immunomodulating and immunoenhancing agent.

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黄芪皂苷Ⅳ对小鼠 T、B 淋巴细胞增殖和腹腔巨噬细胞功能的影响[1]

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关键词 膜荚黄芪；皂苷类；T-淋巴细胞；B-淋巴细胞；抗体生成；腹腔巨噬细胞；白介素-1；肿瘤坏死因子

目的：探讨黄芪皂苷Ⅳ（ASI）对小鼠 T、B 淋巴细胞增殖和腹腔巨噬细胞分泌的细胞因子的影响。**方法**：采用 MTT 法检测 T、B 淋巴细胞的增殖；采用分光光度计测定法检测抗体活性；IL-1 活性用胸腺细胞增殖法测定；TNF-α 活性用 L929 细胞杀伤法测定。**结果**：1）ASI 50-200 mg/kg ig 7 天能够促进 T 淋巴细胞增殖和抗体生成，而 ASI 50-100 mg/kg 能够促进 B 淋巴细胞增殖，但是 200 mg/kg 对 B 淋巴细胞增殖无影响；2）ASI 体外仅在 100 nmol/L 对 T、B 淋巴细胞有促进作用；3）ASI 1 nmol/L 可以促进腹腔巨噬细胞分泌 IL-1，而 100-1000 nmol/L 则抑制腹腔巨噬细胞分泌 IL-1；4）ASI 体外可以抑制 LPS 刺激或无 LPS 刺激下的腹腔巨噬细胞分泌 TNF-α。**结论**：ASI 能够促进小鼠 T、B 淋巴细胞的增殖和抗体生成，同时可以抑制腹腔巨噬细胞体外分泌 IL-1 和 TNF-α。

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