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

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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 hemolysis 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. Its chemical components including polysaccharides, flavonoids, and saponins. The immunomodulatory effects of Am crude extracts and its components were reported. 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). Astragaloside IV (ASI) is a pure saponin isolated from radix of Am, which is a diglycoside of cycloastragenol possessing one glucoside moiety and one xyloside moiety. 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.

MATERIALS AND METHODS

Astragalosides Astragalosides (AST) is the total saponin fraction isolated from Shanxi Astragalus membranaceus (Fisch.) Bge by MeOH extract and γ-BuOH distribution. AST is a white powder, ground, and suspended in 0.1% CMC.
ASI is colorless needles crystallized from AST by MeOH, mp 299 – 301 °C, 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 I, Certificate No 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 Concanaevalin (ConA, No 27700) and lipopolysaccharides (LPS, No 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 mmol/L, and 10 % fetal bovine serum, pH 7.2. Crystal violet was supplied by Shanghai Chongming Chemical Reagent Factory.

Lymphocyte proliferation assay Mouse spleen was torn and passed through a stainless steel (100 μ). The single cell suspension was adjusted to 5 × 10^6/μL. 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 °C, 5 % CO₂ 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 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 × 10^6/L, incubated with sheep erythrocyte and guinea pig serum 1 mL respectively at 37 °C 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 IL-1 production was measured by thymocyte proliferation assay. Suspension of thymocyte 100 μL (2 × 10^6/L) taken from BALB/c mice was co-cultivated 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 °C 5 % CO₂ incubator. MTT 20 μL was added and incubated for 4 h. Absorbance was determined at 570 nm by microplate spectrophotometer.

TNF-α assay TNF-α activity was determined by the cytotoxicity assay against L929 cells as described previously. L929 cells (4 × 10^3/L) were incubated for 24 h to form a single-layered cell. The 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 °C 5 % CO₂ 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:

% Cytotoxicity = (A_control – A_test) / A_control × 100 %

Statistics The data were expressed as x ± 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. x ± s. P < 0.05, P < 0.01 vs control. Absorbance for antibody: A₅₅₂ nm; for T, B lymphocyte: A₄₉₀ nm.

Tab 1. Effect of ASI on T, B lymphocyte proliferation in vitro. n = 3. x ± s. P < 0.05 vs control group.

<table>
<thead>
<tr>
<th>Concentration/ nnol·L⁻¹</th>
<th>A₅₅₂ nm</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>0 (Control)</td>
<td>0.15 ± 0.03</td>
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<tr>
<td>0.1</td>
<td>0.133 ± 0.013</td>
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<tr>
<td>1</td>
<td>0.160 ± 0.010</td>
</tr>
<tr>
<td>10</td>
<td>0.160 ± 0.010</td>
</tr>
<tr>
<td>100</td>
<td>0.147 ± 0.015</td>
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<tr>
<td>1000</td>
<td>0.16 ± 0.03</td>
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Tab 2. Effect of ASI on IL-1 production from peritoneal macrophages in vitro. n = 3. x ± s. P < 0.05 vs control.

<table>
<thead>
<tr>
<th>Concentration/ nnol·L⁻¹</th>
<th>A₄₉₀ nm</th>
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<tr>
<td></td>
<td></td>
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<tr>
<td>0 (Control)</td>
<td>0.49 ± 0.03</td>
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<tr>
<td>0.1</td>
<td>0.48 ± 0.07</td>
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<tr>
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<tr>
<td>100</td>
<td>0.393 ± 0.010</td>
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<td>1000</td>
<td>0.393 ± 0.010</td>
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DISCUSSION

Am is a traditional herb used in China with long history. The components of Am have been reported to enhance immune function in mice⁹. 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 arthritis¹⁰. 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 vitro¹¹. 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 vivo¹², 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...
proliferation and antibody production, but inhibited IL-1 and TNF-α production from peritoneal macrophages at high concentration in vitro, indicating that ASI is a potent immunomodulating and immunoenhancing agent.

REFERENCES


