Production of Th1- and Th2-dependent cytokines induced by the Chinese medicine herb, *Rhodiola algida*, on human peripheral blood monocytes

H.X. Li a, S.C.W. Sze a, Y. Tong a,*, T.B. Ng b

a School of Chinese Medicine, LKS Faculty of Medicine, The University of Hong Kong, 10 Sassoon Road, Pokfulam, Hong Kong, China
b Department of Biochemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China

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**A B S T R A C T**

*Ethnopharmacological relevance:* *Rhodiola algida*, an herb ingredient used in Chinese medicine, has been clinically proven to be effective in enhancing human immune responses.

**Aim of study:** This study attempted to identify the potential immunomodulatory effect of *Rhodiola algida* extract in human immune system in vitro, and to examine its underlying molecular effects.

**Materials and methods:** Firstly, the bioactive marker compound salidroside was used for standardization of *Rhodiola algida* extract by reversed-phase HPLC. Secondly, the regulation of human immune responses was investigated in human peripheral blood monocytes. A series of cytokines known to play important roles in the human immune responses were examined.

**Results:** The current study provided quantitative assay for the marker compound, salidroside, in the *Rhodiola algida* extract by reversed-phase HPLC. Secondly, the regulation of human immune responses was investigated in human peripheral blood monocytes. A series of cytokines known to play important roles in the human immune responses were examined.

**Conclusion:** The findings may enable us to further explain the pharmacological properties in Chinese medicine and make *Rhodiola algida* a very promising immunomodulating agent.

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1. Introduction

The genus *Rhodiola* L. (Crassulaceae) consists of nearly 200 species in which 20 species are commonly used in traditional medical practice in Eastern Europe and Asia (Germano et al., 1999; Kelly, 2001). Of these 20 species, *Rhodiola algida*, *Rhodiola rosea*, *Rhodiola sachalinensis*, *Rhodiola kirilowii*, *Rhodiola crenulata*, *Rhodiola stropaurea*, *Rhodiola gundrifida*, and *Rhodiola serba* are the most effective ones. In China, *Rhodiola crenulata* H. Ohba is the only authorized herb according to Chinese Pharmacopoeia. However, many other species are also popular in folk medicines such as *Rhodiola algida*. *Rhodiola* is a Tibetan plant, growing in damp habitats on highlands near the snow line on the mountains. It is distributed in the southwest of China, Altai Mountains, Central Siberia and Mongolia, 3000 m above the sea level (Mishra et al., 2006). Its lethal temperature is –26.5 °C. The medicinal use of the plant is usually concentrated in the roots and rhizomes that contain its main bioactive compounds (Wang et al., 2006).

*Rhodiola* L. has been widely used in traditional Chinese medicine for a long time (Germano et al., 1999; Kelly, 2001). As a traditional herbal remedy, *Rhodiola* has been used by Chinese practitioners in many ways, such as promoting blood circulation, cleaning heat in the lungs, eliminating toxins from the body, and treating various endemic diseases (Lishmanov et al., 1993; Maimeskulova et al., 1997; Maimeskulova and Maslov, 2000). *Rhodiola* plants are prescribed to increase physical endurance, work productivity, longevity, resistance to high latitude sickness, and to treat fatigue, depression, nervous system disorders, etc. (Arora et al., 2005). Recent development has shown that *Rhodiola* plants have potent adaptogenic, cardioprotective, hepatoprotective, and immunomodulating activities (Song et al., 2003).

With the dramatical increase in demand for *Rhodiola*-based phyto-medicines in the late 1980s, and because of their significant species-dependent variations in phytochemistry, the standardization of *Rhodiola* products or extracts is of great importance. For example, different *Rhodiola* L. species and products had been analyzed using high-performance liquid chromatography (HPLC) methods (Ganzera et al., 2001; Tolonen and Usitalo, 2004). More than 30 chemical compounds have been characterized from the...
Rhodiola plants (Luo et al., 2005). Salidroside is regarded as the most important bioactive component and has been used extensively as an indicator for quality evaluation of many Rhodiola species and products (Cui et al., 2003). Therefore, salidroside was selected in this study as a standard marker to evaluate the quality consistency among different batches of Rhodiola algida.

Rhodiola algida is one of the most effective species of Rhodiola L. and has been clinically proven to enhance human immune responses (Germano et al., 1999; Kelly, 2001). Despite these findings, the role of Rhodiola algida as an immunomodulatory agent has not been established. This study aimed to identify and explore the potential immunomodulatory effects and the underlying mechanisms of the chemically standardized Rhodiola algida extract in human peripheral blood lymphocytes.

2. Materials and methods

2.1. Herbal materials

The raw materials of Rhodiola algida used for these studies were originally produced in Yunnan province, People’s Republic of China, and packaged by Guangzhou Xueyu Company Ltd. (Guangzhou, China). They had been tested by SGS Hong Kong Limited (Société Générale de Surveillance) for the presence of heavy metals and microorganisms, and found to be above the international safety level and free from microorganisms, respectively.

2.2. Preparation of Rhodiola algida and quality analysis

The herb was first boiled in distilled water at 100 °C for 1.5 h. The aqueous herbal extract was further concentrated to dryness in vacuum at room temperature, followed by heating at 60 °C until it became a solid residue. The dried residue was stored in a dehydra-
tion chamber until use.

For evaluation of the quality consistency among Rhodiola algida extracts, six batches of Rhodiola algida extracts (200 mg) were weighed and extracted with 10 mL of methanol, followed by ultrasonic for 30 min. After centrifugation, the supernatant was filtered by a 0.45-μm Milli^3 syringe filter unit, and then injected in a volume of 10 μL in HPLC. A standard chemical of salidroside, which is a well-known constituent of Rhodiola algida (Cui et al., 2003), was employed and compared to the fingerprints of different batches of Rhodiola algida extract.

Reproducibility and linearity were estimated by performing repetitive injections. The external standard method, using a series of standard solutions with concentrations ranging from 12.5 to 200 μg/mL, was needed. A reversed-phase column (XBridge® C18, 5 μm, 150 mm × 3.9 mm i.d., Thermo, USA) was used and the mobile phase consisted of a binary mixture of methanol and water buffer (15:85, v/v). The flow rate was 1.0 ml/min. DAD detector was set at 275 nm for obtaining chromatograms with the maximum number of peaks. Chromatogram and peak integration were analyzed by the software of Millennium® Chromatography Manager Version 3.2.

2.3. Drug preparation, sample collection and isolation of the blood mononuclear cells

The Rhodiola algida extract was weighed and then dissolved in dimethyl sulphoxide (DMSO) at a concentration of 100 mg/mL. For use, the Rhodiola algida extract was diluted (10 mg/mL) in Hank’s Balanced Salt Solution (HBSS) (Invitrogen, Carlsbad, CA, USA) and filtered using 0.22-μm microcellulose acetate (Corning, NY, USA) for sterilization. The solution (10 mg/mL) was further diluted with supplemented RPMI-1640 medium to various concentrations (10, 50, 100, and 200 μg/mL) for the following experiments. The various concentrations of the extract were freshly prepared before use.

The ethics of human blood acquisition and subsequent use was approved by Institutional Review Board, the University of Hong Kong. Adults of both sexes (48 males and 32 females) with age from 22 to 59 (average age 39.94) were recruited for this study. Informed consent was obtained from all participants before the experiments. A total of 80 healthy adult volunteers of both sexes were recruited by consecutive sampling during a specified period on a random-
ized base. Their peripheral blood samples (10 mL) were collected in sterilized columns containing heparin from the Department of Surgery, Queen Mary Hospital, The University of Hong Kong. Fresh blood samples were used within 1 h after collection.

Isolated peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-Paque density gradient (GE Healthcare, Sweden). Heparinised peripheral blood (10 mL) was diluted with 10 mL of HBSS. Ficoll-Paque PLUS (15 mL) was added to a 50-ml centrifuge tube, and then 20 mL of diluted blood sample was carefully layered on the Ficoll-Paque PLUS. The mixture was centrifuged at 1500 rpm for 30 min at 20 °C. Then the upper layer was withdrawn using a clean Pasteur pipette, leaving the lymphocyte layer undisturbed at the interface. The lymphocyte layer was transferred using a clean Pasteur pipette to a clean centrifuge tube. HBSS (15 mL) was added to the lymphocytes in the test tube. The cells were suspended by gently drawing them in and out of a Pasteur pipette. The cells were centrifuged at 1800 rpm for 10 min at 20 °C. The supernatant was removed. The cells were washed three times as described above. The lymphocytes were maintained in RPMI-1640 medium, supplemented with heat-inactivated 10% fetal bovine serum (FBS) and 1% antibiotics (100 U penicillin and 100 μg/mL streptomycin).

Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO2 for 1 h to remove adherent macrophages. Peripheral blood mononuclear cells 2 (PBMC) were then collected and maintained in supplemented RPMI-1640 medium at 37 °C in a humidified atmos-
phere containing 5% CO2.

2.4. Cell proliferation assay and colony formation assay

In order to verify a possible cell proliferation effect, increasing doses of Rhodiola algida extract were added to PBMC cell suspension. PHA, as a mitogenic lectin, was shown to induce human lymphocytes proliferation and cytokine release (Suvachittanont and Jaranchavanapat, 2000). Preliminary clinical data from our group had shown that the optimal stimulating concentration of PHA on human peripheral lymphocytes was 20 μg/mL (unpublished data). Therefore PHA at the concentration of 20 μg/mL was added to medium as a positive control. Medium alone was a negative control.

Cell proliferation was assessed in vitro using the tetrazolium compound WST-1 (Roche, Indianapolis, IN, USA). The lymphocytes were cultured at a density of 5 × 10^6 cells/mL into 96-well plates. Cell growth was followed for 24, 48 and 72 h after stimulation in the presence or absence of various concentrations of Rhodiola algida extracts (10, 50, 100, 200 μg/mL). At 24, 48, and 72 h of the treat-
ment, the cells were collected and washed three times with HBSS, and 100 μL of a 1:10 dilution of WST-1 reagent was added according to the manufacturer’s instructions (Roche, Indianapolis, IN, USA). The cells were incubated for 1 h at 37 °C, and the absorbance was read at 450 nm for each well on a spectrophotometer (Molecu-
lar Devices Corp., CA, USA). The increased absorbance is directly proportional to cell viability.

The cells were plated onto 24-well plates at an initial cell concentration of 5 × 10^3 cells/well. Fresh medium was added with or without extract of Rhodiola algida (50 μg/mL) for 72 h. PHA (20 μg/mL) was added as a positive control. At the end of the treatment, 500 μL of the cell suspensions were transferred onto a specimen chamber (Wescor Inc., Logan, USA). The chamber was fixed onto a holder equipped with a membrane filter, and cen-
trifuged using a cytocentrifuge (Wescor Inc., Logan, USA). The filter was recovered from the chamber and air-dried. The cells retained on the filter were fixed with acetone for 10 min, and stained with eosin and hematoxylin (H&E). The slides were then hydrated consecutively into different concentrations of ethanol (70%, 95%, and 100%) and finally immersed in xylene for 3 min, respectively. The slides were mounted and visualized under the 200× microscope (Leica DME, NY, USA).

2.5. Metabolic rate assay (ATP bioluminescence assay) and DNA determination

After incubation with herbal extract or with PHA for 72 h at the initial concentration of 1 × 10^6 cells/well, cells were transferred to microcentrifuge tubes and 0.5 mL of cell lysis reagent was added to the cells. In preparation of the luciferase reagent of the ATP bioluminescence assay, 10 mL of dilution buffer was added to the bottle of luciferase reagent supplied by the kit (Promega, Madison, USA). Cell lysis was accelerated by a sonicator (Sonics & Materials Inc., Danbury, CT, USA) at a pulse of 30 min⁻¹ with 5% amplitude, for 1 min. There was a 10-s binding period preceded by amalgamation of 50 μL of samples and 50 μL luciferase reagent. The absorbance of the samples was read by using a TD-20/20 Luminometer (Turner Designs, CA, USA) at the wavelength of 420 nm. Optical densities of the samples should be converted from RLU to ATP mass using an ATP standard curve. A standard ATP curve was prepared with materials supplied by the kit, whose working range was between 10⁻¹⁵ and 10⁻¹⁰ moles of ATP. ATP activity of the cells was expressed as unit per μg of DNA as there was variation in size of the cell number cultured in each well. The amount of DNA was then measured by using a fluorometer (Turner Biosystems, Sunnyvale, CA, USA).

2.6. Enzyme-linked immunosorbent assay (ELISA)

For ELISA analyses of cytokines, PBMC at initial concentration of 2 × 10⁶ cells/well were treated with *Rhodiola alpida* or PHA for 72 h. After the treatment, the cells were harvested and centrifuged at 2000 rpm for 10 min. The supernatants were removed for ELISA assays. Cytokine levels in the culture supernatants were determined using IL-2, IL-4, IL-8, IL-10, TNF-α and GM-CSF ELISA kits (Diaclone, Stamford, CT, USA) according to the manufacturer’s instructions. Detailed information about these ELISA’s kits was provided in Table 1. The concentrations of cytokines in each sample were calculated from a standard curve prepared using known concentrations of recombination cytokines.

2.7. RNA isolation and reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from cultured PBMC cells by using Geneaid® DNA Mini Kit (cultured cells) (Geneaid, Taiwan) according to the manufacturer’s instructions. The quantity and quality of the extracted RNA was confirmed by absorption measurements at 260 and 280 nm using a spectrophotometer (U-1800, HITACHI, Tokyo, Japan). RT-PCR was performed using the SuperScript kit (Life Technologies, Rockville, MD, USA). RNA extraction was synthesized according to the manufacturer’s instructions. Briefly, 3 μg of RNA was mixed with oligo (dT) (50 μM) as reverse primer, and nuclelease-free water was added to a final volume of 10 μL. The RNA/primer mixture was incubated at 65°C for 5 min and chilled at 4°C for at least 1 min. The RT mixture was prepared following the manufacturer’s protocol and added to the chilled RNA/primer mixture. After incubation at 50°C for 50 min and 85°C for 5 min, two units of RNase H were added to the reaction, and the reaction mixture was incubated at 37°C for 20 min. The products were stored at –80°C for the following experiments. PCR reaction was performed in 50 μL reaction volume. Primer sequences used for PCR amplification were synthesized by Molecular Informatrix Laboratory (Tech Dragon Ltd., Hong Kong). Conditions of PCR mixture were: 25 nmol of each primer, 220 μM each of dGTP, dATP, dCTP and dTTP, 55 mM KCl, 22 mM Tris–HCl, pH 8.4, 1.65 mM MgCl₂, 1 U Taq DNA polymerase. 2.5 μL of first strand cDNA was added to the PCR reactions. Reactions were incubated in a PTC-200 Thermal Cycler (MJ Research, Watertown, MA, USA). The PCR primer sequences are shown in Table 2. Cycles and temperatures for each gene are shown in Table 3. PCR products were run on a 2% agarose gel followed by ethidium bromide staining and capturing in a gel documentation system (Chemi Genius Image Acquisition System, Syngene, Cambridge, UK). Signals were quantified using densitometric analysis software (NIH Image 1.32; National Institutes of Health, Bethesda, MD, USA). Data are expressed as the signal of the ratio obtained for

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The detection limit of cytokines using ELISA kits.</th>
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<tbody>
<tr>
<td>ELISA kit</td>
<td>Detection limit</td>
</tr>
<tr>
<td>h IL-2</td>
<td>31.2–1000 pg/mL</td>
</tr>
<tr>
<td>h IL-4</td>
<td>1–35 pg/mL</td>
</tr>
<tr>
<td>h IL-8</td>
<td>62.5–2000 pg/mL</td>
</tr>
<tr>
<td>h IL-10</td>
<td>12.5–400 pg/mL</td>
</tr>
<tr>
<td>h TNF-α</td>
<td>25–800 pg/mL</td>
</tr>
<tr>
<td>h GM-CSF</td>
<td>31.25–1000 pg/mL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Primer sequences used for the detection of gene expression of cytokines.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
<td>Directions</td>
</tr>
<tr>
<td>IL-1α</td>
<td>Forward</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Reverse</td>
</tr>
<tr>
<td>IL-2</td>
<td>Forward</td>
</tr>
<tr>
<td>IL-4</td>
<td>Reverse</td>
</tr>
<tr>
<td>IL-6</td>
<td>Forward</td>
</tr>
<tr>
<td>IL-8</td>
<td>Reverse</td>
</tr>
<tr>
<td>IL-10</td>
<td>Forward</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Reverse</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Forward</td>
</tr>
<tr>
<td>β-Actin</td>
<td>Reverse</td>
</tr>
</tbody>
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<table>
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<tr>
<th>Table 3</th>
<th>PCR conditions used in this study.</th>
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</thead>
<tbody>
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<td>Gene</td>
<td>Product size (bp)</td>
</tr>
<tr>
<td>IL-1α</td>
<td>469</td>
</tr>
<tr>
<td>IL-1β</td>
<td>667</td>
</tr>
<tr>
<td>IL-2</td>
<td>455</td>
</tr>
<tr>
<td>IL-4</td>
<td>317</td>
</tr>
<tr>
<td>IL-6</td>
<td>425</td>
</tr>
<tr>
<td>IL-8</td>
<td>458</td>
</tr>
<tr>
<td>IL-10</td>
<td>352</td>
</tr>
<tr>
<td>TNF-α</td>
<td>451</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>387</td>
</tr>
<tr>
<td>β-Actin</td>
<td>539</td>
</tr>
</tbody>
</table>
Fig. 1. HPLC chromatogram of salidroside from *Rhodiola algida* extract monitored at the wavelength of 275 nm. The inset is DAD UV scan of salidroside peak (275 nm).

Each gene in one sample divided by that obtained for the housekeeping gene (β-actin) in the same sample.

2.8. Statistical analysis

Simple descriptive summary statistics were used to describe the morphologic characteristics of the studied samples. Statistical analysis was performed using SPSS 11.0 (SPSS, Inc., Chicago, IL, USA) including the ANOVA test for pairwise comparisons. The continuous variables were analyzed by independent sample t-test. All P-values reported were two-sided with P < 0.05 considered significant. Experimental results were presented as the mean ± standard deviation.

3. Results

3.1. Quality control

For exploring most of detectable peaks in the HPLC chromatogram, the spectra of all eluted peaks in the chromatogram of *Rhodiola algida* extracts were investigated with photodiode array detection. The chromatograms were generated under the detection wavelength of 275 nm. A chromatographic fingerprint showing the elution peaks of standard compounds, salidroside, and other common peaks is shown in Fig. 1. Six batches of *Rhodiola algida* extracts were examined by HPLC using the optimum running conditions. The results of their contents are shown in Table 4. Inter-assay relative standard deviation (R.S.D.) values were less than 5%.

3.2. Effects of herbal extract on proliferation of lymphocytes

PBMC treated with the extract of *Rhodiola algida* exhibited differential growth responses (Fig. 2). Cell proliferation was stimulated after treatment with 10, 50, 100 and 200 μg/mL of *Rhodiola algida* extract induced a time- and dose-dependent stimulation of cell growth in PBMC. After treatment for 72 h with 50 μg/mL of *Rhodiola algida* extract cell growth was stimulated by twofold. The effect is equipotent to PHA. Hence, the 50 μg/mL concentration of *Rhodiola algida* extract was selected as the concentration for the subsequent experiments.

3.3. Assay of metabolic rate

It is known that the nucleotide 5′-adenosine triphosphate (ATP) is released constitutively from cells and in direct response to environmental stress (Chi and Pizzo, 2006). Thus, ATP content or energy charge may be a good parameter to assess cell viability. As shown in Fig. 3, ATP values in human PBMC cells stimulated with either PHA (20 μg/mL) or *Rhodiola algida* extract (10, 50 and 100 μg/mL) was significantly higher than the control group (P < 0.01, 0.05, 0.01, and 0.05, respectively). Results obtained from this study indicated that *Rhodiola algida* extract can increase the metabolic rate of human PBMC in vitro.

![Fig. 2. Time- and dose-dependent effects of *Rhodiola algida* extract (RHO) on growth of human PBMC (n = 80). PBMC were plated onto 94-well plates and treated with varying concentrations (10, 50, 100 and 200 μg/mL) of *Rhodiola algida* extract for 24, 48, and 72 h. Phytohemagglutinin (PHA, 20 μg/mL) alone was added as the positive control. No RHO or PHA was used in control (i.e. negative control). Cell proliferation was determined by the WST-1 assay. Results (optical densities) are expressed as mean ± standard deviation. Different alphabets above bars indicate statistically significant (P < 0.05) difference between the data when the data are subjected to analysis of variance followed by Duncan’s multiple range test.](image-url)
Fig. 3. Dose-dependent effects of Rhodiola algida extract (RHO) on ATP concentration released from human PBMC (n = 80). PBMC were plated onto 96-well plates and treated with varying concentrations (10, 50, 100 and 200 µg/mL) of Rhodiola algida extract for 72 h. PHA (20 µg/mL) alone was added as the positive control. Results are expressed as mean ± standard deviation. Different alphabets above bars indicate statistically significant (P < 0.05) difference between the data when the data are subjected to analysis of variance followed by Duncan’s multiple range test.

3.4. Assay of colony formation

To investigate the influence of Rhodiola algida extract on transformed lymphocyte independent growth, clonogenic assay was examined under the microscope by staining with H&E. The morphology of lymphocytes aggregation measured before and after stimulating by Rhodiola algida extract is shown in Fig. 4. At day 0, there were scattered lymphocytes and no colony formation was observed (Fig. 4A). After treatment with Rhodiola algida extract (50 µg/mL) for 72 h, the cells showed a significantly increase in the cell aggregation (Fig. 4C and D). The effect of Rhodiola algida extract is comparable to that of PHA (20 µg/mL). The cells treated with media alone showed no significant changes in the colony formation (Fig. 4B).

3.5. Activities and expression of interleukins

To determine the effectiveness of Rhodiola algida extract on the human lymphocytes, the activities of interleukins (IL-2, IL-4, IL-8, and IL-10) were analyzed by ELISA and RT-PCR. Gene expression was quantified and expressed as the ratio of the signals obtained for each gene in one sample divided by that obtained for the house-keeping gene (β-actin) in the same sample.

Total soluble interleukin activities measured in the cell culture supernatants after incubation for 72 h, with or without Rhodiola algida extracts are shown in Figs. 5A–8A. After incubation for 72 h, it was observed that, as compared with the negative control group, PHA induced a significant increase in IL-2 (Fig. 5A), IL-4 (Fig. 6A), IL-8 (Fig. 7A), and IL-10 levels and activities (Fig. 8A) (P < 0.05, 0.01, 0.01 and 0.05, respectively). As for Rhodiola algida, a significant increase in IL-2, IL-4, IL-8, and IL-10 levels (Figs. 5–8) (P < 0.05, 0.05, 0.01, and 0.05, respectively) was also observed. The stimulating effects of Rhodiola algida extract on these four cytokines were similar to that of PHA. Quantitative analysis by RT-PCR of the gene expression of the interleukin IL-2, IL-4, IL-8, and IL-10 (Figs. 5B–8B) confirmed the up-regulation of these four genes in human PBMC cells. In addition, after incubation with Rhodiola algida extract for 72 h, a significant increase in IL-6 (Fig. 9) mRNA expression was induced in human PBMC cells.

3.6. TNF-α and IL-1 activities and expression

Though IL-1 and TNF-α are different in structure and effectors, their spectra of biological effects overlap considerably. Both can help to initiate humoral and cellular immune responses (Shevach, 2006). Since Rhodiola algida extract stimulated proliferation of human lymphocyte, we further investigated its ability to induce soluble levels and gene expression of IL-1 (IL-1α and IL-1β) and TNF-α in human PBMC cells. After incubation for 72 h, both Rhodiola algida extract and PHA induced significant increase in TNF-α
Fig. 5. *Rhodiola algida* extract induces increase in IL-2 secretion and IL-2 gene expression in human lymphocytes. (A) IL-2 concentration (pg/mL) in culture supernatants of PBMC incubated with *Rhodiola algida* or PHA for 72 h. (B) IL-2 mRNA levels in PBMC incubated with *Rhodiola algida* for 72 h. RT-PCR was performed using IL-2 and β-actin primers. Data are presented as the ratio between IL-2 and β-actin mRNA levels. Agarose gel results are representative of two similar experiments. Results are expressed as mean ± standard deviation. Different alphabets above bars indicate statistically significant (P < 0.05) difference between the data when the data are subjected to analysis of variance followed by Duncan’s multiple range test.

levels in cell culture supernatants (P < 0.01 and 0.05, respectively) (Fig. 10A). They also significantly augmented in expression of TNF-α, IL-1α and IL-1β mRNA in human PBMC cells (Figs. 10B and 11).

3.6.1. GM-CSF activity and expression

Normal human PBMC cells, particularly T lymphocytes, are a rich source of granulocyte-macrophage colony-stimulating factor (GM-CSF) (Chikkappa et al., 1998; Fleetwood et al., 2005). Release of GM-CSF from T cells is important in the differentiation, maturation, and survival of inflammatory cells (Fleetwood et al., 2005). PHA has been confirmed as a mitogenic agent and can stimulate PBMC cells to produce as much as 100-fold increase in GM-CSF (Chikkappa et al., 1998). As *Rhodiola algida* showed a significant effect on the growth of PBMC, we further investigated the stimulating effect of *Rhodiola algida* on GM-CSF. PHA was used as a positive control. Soluble GM-CSF activities measured at 72 h after treatment with *Rhodiola algida* extract are shown in Fig. 12A. Results showed that PHA increased GM-CSF levels by 100% and the finding was consistent with the previous reports (Chikkappa et al., 1998). Statistical analysis indicated that GM-CSF activities in human PBMC cells treated with *Rhodiola algida* extract was significantly increased as compared to the control group, suggesting that both *Rhodiola algida* extract and PHA could increase GM-CSF activity (P < 0.01). Quantitative assay of the GM-CSF mRNA expression also showed that *Rhodiola algida* up-regulated the gene expression of GM-CSF in human PBMC cells (Fig. 12B).

4. Discussion

In this study, a novel, simple, accurate, reliable and reproducible method to evaluate the quality consistency of *Rhodiola algida* among different batches was developed by using the well-known compound salidroside as a marker in a single chromatographic run at the detection wavelength of 275 nm (Fig. 1). A standard chemical of salidroside, which is a well-known compound in *Rhodiola algida* (Cui et al., 2003), was employed and compared to the fingerprint of *Rhodiola algida* extract. This chromatographic fingerprint with salidroside as a marker compound is used as a reference standard, indicating the purity, identity and quality consistency among the *Rhodiola algida* extracts. Salidroside, but not the additional salidroside-like glycoside compounds (rhodiolin, rosin, rosavin, rosarin, and rosiridin), is regarded as the most important bioactive component and has been used as an indicator for the quality control of different *Rhodiola* species (Cui et al., 2003). Salidroside has been found in all studied species of *Rhodiola* including *Rhodiola algida* while other active glycosides, such as rosavin, rosin, and rosarin, are just scattered in different *Rhodiola* species (Linh et al., 2000). Because of this variation within the *Rhodiola* genus, verifica-
Fig. 7. *Rhodiola algida* extract induces increase in IL-8 secretion and IL-8 gene expression in human lymphocytes. (A) IL-8 concentration (pg/mL) in culture supernatants of PBMC incubated with *Rhodiola algida* or PHA for 72 h. (B) IL-8 mRNA levels in PBMC incubated with *Rhodiola algida* for 72 h. RT-PCR was performed using IL-8 and β-actin primers. Data are presented as the ratio between IL-8 and β-actin mRNA levels. Agarose gel is representative of two similar experiments. Results are expressed as mean ± standard deviation. Different alphabets above bars indicate statistically significant (P<0.05) difference between the data when the data are subjected to analysis of variance followed by Duncan’s multiple range test.

Fig. 8. *Rhodiola algida* extract induces increase in IL-10 secretion and IL-10 gene expression in human lymphocytes. (A) IL-10 concentration (pg/mL) in culture supernatants of PBMC incubated with *Rhodiola algida* or PHA for 72 h. (B) IL-10 mRNA levels in PBMC incubated with *Rhodiola algida* for 72 h. RT-PCR was performed using IL-10 and β-actin primers. Data are presented as the ratio between IL-10 and β-actin mRNA levels. Agarose gel is representative of two similar experiments. Results are expressed as mean ± standard deviation. Different alphabets above bars indicate statistically significant (P<0.05) difference between the data when the data are subjected to analysis of variance followed by Duncan’s multiple range test.

Fig. 9. *Rhodiola algida* extract induces increase in IL-6 expression in human PBMC. IL-6 mRNA levels in PBMC incubated with *Rhodiola algida* for 72 h. RT-PCR was performed using IL-6 and β-actin primers. Data are presented as the ratio between IL-6 and β-actin mRNA levels. Agarose gel is representative of two similar experiments.

The human immune system is important because it has a close involvement in a lot of diseases such as allergy, arthritis, cancer and AIDS, etc. (Romagnani, 1997, 2004). Lymphocytes are small white blood cells that bear the major responsibilities of carrying out the activities of the immune system (Beissert et al., 2006). Our study has...
first investigated the effects of *Rhodiola algida* extract on expression of both Th1 and Th2 cytokines from human peripheral lymphocytes. Results showed that *Rhodiola algida* extract stimulated proliferation and metabolic rate of human lymphocytes by 100% after treatment for 72 h. The underlying mechanism might be through stimulating the expression of IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α and GM-CSF in human PBMC cells. The study suggests that *Rhodiola algida* is a potential immunomodulator through its mediation of both humoral and cellular immunity.

IL-2 plays an important role in promoting T-cell proliferation, cytokine production, and the function properties of B cells, macrophages, and NK cells (Blachere et al., 2006). It was observed in this preliminary study that IL-2 activity and expression were up-regulated after incubation for 72 h with *Rhodiola algida* extract. Our findings were partly in line with those from other studies (Kormosh et al., 2006). In recent investigations, formulas containing different species of *Rhodiola* induced a significant increase in the NK cell activity, IL-2 activity (Mishra et al., 2006). The percentage of peripheral T lymphocyte was increased significantly after stimulation with *Rhodiola* extracts (Kormosh et al., 2006). Paradoxically, however, this cytokine appears to be equally important in limiting such responses and eliminating autoreactive T cells (Hashimoto et al., 2006). IL-2 is thus a two-edged weapon that initiates immune responses and eliminating autocreative T cells (Hashimoto et al., 2006). It appears to be equally important in limiting IL-2 activity and expression were up-regulated after incubation for 72 h with *Rhodiola algida* extract.

**Fig. 10.** *Rhodiola algida* induces increase in TNF-α secretion and TNF-α gene expression in human lymphocytes. (A) TNF-α concentration (pg/mL) in culture supernatants of PBMC incubated with *Rhodiola algida* or PHA for 72 h. (B) TNF-α mRNA levels in PBMC incubated with *Rhodiola algida* for 72 h. RT-PCR was performed using TNF-α and β-actin primers. Data are presented as the ratio between TNF-α and β-actin mRNA levels. Agarose gel is representative of two similar experiments. Results are expressed as mean ± standard deviation. Different alphabets above bars indicate statistically significant (P < 0.05) difference between the data when the data are subjected to analysis of variance followed by Duncan’s multiple range test.

**Fig. 11.** *Rhodiola algida* induces increase in IL-1α and IL-1β expression in human PBMC. (A) IL-1α mRNA levels in PBMC incubated with *Rhodiola algida* for 72 h. (B) IL-1β mRNA levels in PBMC incubated with *Rhodiola algida* for 72 h. RT-PCR was performed using IL-1α or IL-1β and β-actin primers. Data are presented as the ratio between IL-1α or IL-1β and β-actin mRNA levels. Agarose gel is representative of two similar experiments.

Th2 cells (Lafreniere et al., 2006). Many studies have demonstrated that IL-4 plays an important role in exacerbating allergic and asthmatic symptoms such as allergic hypersensitivity (Lafreniere et al., 2006). IL-6 is a pleiotropic cytokine that influences antigen-specific immune responses and inflammatory reactions (Kishimoto, 2006). It has been found to play a central role in defense mechanisms, regulating the immune responses, hematopoiesis and immune-mediated inflammatory diseases (Smolen and Maini, 2006). IL-10 is a product of numerous cells but mainly secreted by Th2 lymphocytes (Beissert et al., 2006). It inhibits the production of IL-2 and IFN-γ by Th1 cells, reduces pro-inflammatory cytokines, downregulates eosinophil function and activity, and suppresses IL-5 production (Konno et al., 2006). Thus IL-10 generates T-cell tolerance (Zhou et al., 2005). Current study showed that *Rhodiola algida* extract induces an increase in both soluble levels and gene expressions of IL-4, IL-6 and IL-10 in human PBMC cells. As IL-4, IL-6 and IL-10 are crucial Th2 cytokines, the findings suggest that *Rhodiola algida* stimulates human immune responses partially by up-regulating humoral immunity.

IL-8 is responsible for inducing chemotaxis and is important in the regulation of the acute inflammatory response (Lockwood et al., 2005). It is synthesized rapidly at local sites of inflammation where it fulfills its function to recruit and activate acute inflammatory cells (Standiford et al., 1990). IL-1 and TNF-α are different in structure and use different receptors, yet their biologic effects overlap considerably (Montag et al., 2006). Both of them directly
promote growth and differentiation of B cells, activate neutrophils and macrophages, and initiate both humoral and cellular immune responses (Moller and Villiger, 2006). Our findings reveal that Rhodiola algida extract consistently up-regulated the expression of IL-1α, IL-β and TNF-α in human PBMC cells. In addition, both IL-1 and TNF-α induce IL-8 mRNA expression by a wide variety of cells after stimulation. Many Chinese herbs are found to induce increase in the secretion of IL-1 in PBMC (Mao et al., 2001). The current study indicated that Rhodiola algida extract markedly increased soluble IL-8 levels and gene expression in human PBMC cells. But whether up-regulation of IL-8 was due to the up-regulation of IL-1 and TNF-α or by the stimulating effect of Rhodiola algida alone awaits elucidation.

GM-CSF is now recognized as a major generator of in vitro granulocyte and macrophage colonies (Bell, 2006). Recent evidence outlines its role as a key mediator in inflammation and autoimmunity, and it is therefore worthy of consideration as a target for anti-inflammatory therapy (Bell, 2006). Thus we tried to explore the effect of Rhodiola algida on GM-CSF expression in human PBMC cells. Our study indicated that Rhodiola algida induces an increase in the soluble levels of GM-CSF and up-regulates its gene expression in human PBMC cells. As GM-CSF is critical in the regulation of T cell immune responses to alter the Th1/Th2 cytokines balance, we can assume that Rhodiola algida extract on the one hand enhances human immune responses; on the other hand, it can also keep the balance between the Th1/Th2 cytokines.

Unlike previous studies that focused on the single side of human immune responses, this study provides a systematic view of clinical application of Rhodiola algida on human immune response. Our findings suggest that Rhodiola algida can stimulate both Th1 and Th2 type responses while keeping a balance between them. As the divergent effects of Th1 and Th2 cells are seen in their association with deleterious immune reactions in humans, emerging evidences of the immunomodulating effects of Rhodiola algida make it a very promising candidate as an immunostimulating agent.

5. Conclusion

The current study provided quantitative assay for the marker compound, salidroside, in the Rhodiola algida extract for ensuring the quality consistency of Rhodiola algida used in the following experiments. After treatment with Rhodiola algida for 72 h, activities and expression of cytokines such as IL-1, IL-2, IL-4, IL-6, IL-8 and IL-10 were all up-regulated remarkably. Combined with findings of up-regulation of TNF-α and GM-CSF, this study suggests that Rhodiola algida can mediate both cellular and humoral immunity via up-regulation of Th1 and Th2 cytokines. In summary, Rhodiola algida is a potential Chinese herb to regulate human immune responses through modulating both Th1 and Th2 immunities. The findings may enable us to further explain the pharmacological properties in clinic and make Rhodiola algida a very promising immunomodulating agent.

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