Short Communication

**Cistanche salsa** extract enhanced IgM production in the human B cell line BALL-1

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**Cistanche salsa** (C.A. Meyer) is a parasitic plant used as an oriental medicinal tonic in Japan. In this study we show that the *C. salsa* extract dialysate (CSD), which was prepared by removing the low-molecular-weight constituents from the extract using a 3,500 Da molecular-weight-cut-off dialysis membrane, enhanced IgM production in the human B cell line BALL-1. At 100 µg/ml, CSD was shown to be able to increase IgM production 1.8-fold with the added effect of slightly inducing cell proliferation. Intracellular IgM production was also increased upon treatment with CSD. These results suggest that the high-molecular-weight constituents in *C. salsa* is able to activate BALL-1 extracellularly and enhance IgM production.

**Key words** B cell, *Cistanche salsa*, high-molecular-weight, IgM.

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**Introduction**

*Cistanche salsa* (C.A. Meyer) is a parasitic plant used as an oriental medicinal tonic in Japan. In China this plant, which is an important traditional medicine, is used for the treatment of kidney deficiency and neurasthenia. The active ingredients are mainly the phenylethanoids and monoterpenes. Phenylethanoids have been shown to have neuroprotective activity in human *in vitro* and mice *in vivo*. Monoterpenes demonstrated an anti-osteoporotic effect. However, other compounds in *C. salsa*, especially the high-molecular-weight (HMW) compounds, have not been studied. We then investigated whether *C. salsa* extract dialysate (CSD) had an effect on the immune system as we did not find any reports describing antibody production relating to this plant in the literature. We report here for the first time that the extract from *C. salsa* is able to enhance the production of IgM in the human B cell line BALL-1.

Normally, IgM is produced during the primary antibody response. Other antibody isotypes are produced after the initial activation of IgM production, and is known as class switching. BALL-1 is a human B cell line originally obtained from the peripheral blood of a patient with acute lymphoblastic leukemia. BALL-1 has been shown to secrete IgM and is Epstein-Barr virus-determined nuclear antigen negative. It has been reported that BALL-1 also secretes interferon-α, tumor necrosis factor (TNF)-α and TNF-β, and other cytokines that stimulates leukemia cell lines. Salvaris et al. have reported that BALL-1 expresses CD20, which is a specific antigen for B cells involved in B cell differentiation pathway.

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**Materials and Methods**

**Preparation of the Cistanche salsa Extract Dialysate (CSD).** One gram of dried *Cistanche salsa* (Uchida Wakanyaku Co., Ltd., Japan) was cut into small pieces and treated with 20 ml of purified water at 50 °C for 30 min. The acquired extract was centrifuged at 2,200 × g for 20 min. The supernatant was first filtered using a sterilized gauze which was then passed through two kinds of filters (0.45 µm and 0.22 µm). The resulting extract was then filtered through a dialysis membrane with 3,500 Da molecular-weight-cutoff (MWCO) (Spectrum Medical Industries, Inc, USA) to remove low-molecular-weight (LMW) constituents at 4 °C. After dialysis, the extract was passed thorough a filter (0.22 µm) again and subjected to freeze-dry. About 25 mg of dry powder was obtained from 1 g of *C. salsa*, and this was used for the experiment.

**Cell culture.** The human B cell line BALL-1 (RCB 0256) was supplied by the RIKEN CELL BANK (Ibaraki, Japan). BALL-1 was cultured in RPMI-1640 medium containing 10 % fetal bovine serum (BioSource International, Inc., USA). Cells were plated in triplicate at 5 × 10^4^ cells/ml in a 24 well plate or 2 ml dish under humidified 5 % CO_2~/95 % air atmosphere at 37 °C. The acquired cells were counted using a cell counter. Cell death was determined by tripian blue dye exclusion.

**ELISA.** The production of IgM was measured by enzyme-linked immunosorbent assay (ELISA). Briefly, microtiter plates were coated with goat anti-human IgM (BioSource International, Inc., USA) in 50 mM carbonate buffer (pH 9.6) and incubated at 37 °C for 1 hr. After the wells were washed with phosphate buffered saline (PBS) containing 0.05 % Tween20 (TPBS) and the wells were

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filled with a blocking solution (PBS containing 1% bovine serum albumin (BSA)) to prevent non-specific reaction. The blocking solution was washed out three times with TPBS. Then the diluted cultured supernatants and standard solution were added and incubated at 37 °C for 1 hr. After the washing with TPBS, horseradish peroxidase-conjugated goat antibody to human IgM (BioSource International, Inc., USA) was added and the plate was incubated at 37 °C for 1 hr. Finally, the wells were washed three times with TPBS and reacted with a substrate solution containing 0.3 mg/ml 2,2′-azino-di-3-ethylbenzthiazolin sulfonic acid (ABTS) diluted in 0.1 M citrate buffer (pH 4.0) containing 0.03% H2O2. Absorbance of the color reaction was measured at 405 nm.

Intracellular IgM production was similarly measured by ELISA. Intracellular solution containing the μ chain was prepared by treating the cells with a cell lysis buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% TritonX-100, 1 mM EDTA, 50 mM NaF, 30 mM Na2P2O7. The cell lysate was remained on ice for 90 min and was then centrifuged at 12,000 × g for 20 min. The obtained supernatant was diluted with 0.1% BSA-PBS then was applied to the prepared ELISA plates.

Statistical analysis. All data are expressed as the mean ± S.D. Statistical significance was analyzed by use of Student's t-test. The value of P<0.01** was considered to be statistically significant.

Results and Discussion

Figure 1 (a) shows the concentration of IgM produced from BALL-1 treated with various concentrations of CSD for 4 days. CSD enhanced the IgM production in a dose-dependent manner. CSD at 10 μg/ml was shown to be able to enhance IgM production. At 100 μg/ml, CSD was able to

![Figure 1](http://example.com/figure1.png)

**Figure 1** Effect of CSD on IgM production in BALL-1.

The cells were cultured in the presence of various concentrations of CSD for 4 days (a) and 100 μg/ml of CSD during a 5 day time period (b). The amount of IgM produced and secreted into cultured supernatants were measured by ELISA. The data shown are means ± S.D. (n=3). Data with an asterisk are significantly different from the values in the non-treated group at P<0.01** or 0.001***.

![Figure 2](http://example.com/figure2.png)

**Figure 2** Effect of CSD on cell growth of BALL-1.

The cells were plated at 5×10⁴ cells/ml in 2ml dish. The number of cells were counted using a cell counter. ○, non-treated; ●, CSD-treated (100 μg/ml). The data shown are means (n=3). Data with an asterisk are significantly different from the values in the non-treated group at P<0.01** or 0.001***.
increase the production of IgM 1.8-fold. The effect of CSD on IgM production and cell growth during a specific time period was also observed. Fig. 1 (b) shows the concentration of IgM produced from BALL-1 cells treated with 100 μg/ml CSD. Even after 1 day, CSD demonstrated significant increased IgM production over control, the ratio of which ranged from 1.5 to 1.9 fold. In Fig. 2, CSD demonstrates a slight but significant ability to induce cell proliferation. After 3 days, CSD-treated cells began to proliferate faster than non-treated cells. However, the ratio of the number of cells between the CSD treated and control remained a constant ~1.2. No difference was observed in the viability of the cells. The effect of CSD on the IgM production was observed earlier than cell growth.

In this study, we used the extract (CSD) from which the LWM compounds were removed using a 3,500 Da MWCO dialysis membrane. Phenylethanoids1(2) and monoterpens,3 which are LMW active constituents that are characteristically contained in C. salsa, are expected to be removed by the dialysis. A water extract of C. salsa, which contains the LMW compounds because it was not filtered through a dialysis membrane, failed to enhance the production of IgM at concentrations just below those known to induce growth inhibition (data not shown). These results suggest that the active constituents in CSD differ from the water extract and act on the cells extracellularly as CSD was prepared using a 3,500 Da MWCO dialysis membrane.

In this study, intracellular IgM production was evaluated by measuring the amount of intracellular μ chain after 4 days of culture (Fig. 3). Intracellular IgM was also shown to be enhanced 1.2-fold. This result shows that CSD can induce the B cell line BALL-1 to become high IgM producing cells. This study suggests that Cistanche salsa can be effective for stimulating the antibody immune response of B cells.

**References**


**Japanese abstract**

ニクショウ（Cistanche salsa C.A. Meyer）は、日本では強壮作用を有する和漢薬として使用されている寄生植物である。本研究では、分子量3,500カットオフの透析膜を使用して得られたニクショウ抽出液の透析物が、ヒトB細胞株であるBALL-1においてIgM産生を増強することを示す。この透析物は、100 μg/g/mlの濃度でIgM産生量を1.8倍に増加させ、このときわずかに細胞増殖誘導を伴っていた。また、この透析物処理により、細胞内のIgM産生量も増加していた。これらの結果より、ニクショウに含まれる高分子成分が、細胞表面において作用することにより、IgM産生を促進することが示唆された。

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