Cistanche salsa Extract Enhanced Antibody Production in Human Lymph Node Lymphocytes

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Summary

Cistanche salsa (C.A. Meyer) G. Beck, Orobanchaceae is a parasitic plant that is used as an oriental medicinal tonic in Japan. In this study, we show that the extract prepared from *C. salsa* enhanced antibody production in human lymph node lymphocytes (LNL). The production of IgM and IgG was shown to be enhanced 5-fold. The extract also induced interleukin-6 receptor (IL-6R) expression on surface immunoglobulin positive B cells in LNL. These results raise a possibility that the antibody production enhanced by the *C. salsa* extract may be related to the involvement of IL-6R expression in B cell activation.

Keywords: B cell, *Cistanche salsa*, human lymph node lymphocytes, IgG, IgM, interleukin-6.

Introduction

Cistanche is a worldwide genus of holoparasitic desert plants in the family Orobanchaceae. The main sources of *Cistanche* are *Cistanche salsa* (C.A. Meyer) G. Beck and *C. deserticola* Y.C. Ma. In Japan, *C. salsa* is used as medicinal tonic. In China, this plant and *C. deserticola* are important traditional medicines used for the treatment of kidney deficiency and neurasthenia. Their active ingredients are mainly phenylethanoids, monoterpenes and polysaccharides. Phenylethanoids from *C. salsa* have been shown to have neuroprotective activity in human cells [1] and in mice *in vivo* [2]. A monoterpene from *C. salsa* showed anti-osteoporotic effect [3]. *In vitro*, arabinogalactan from *C. deserticola* showed enhanced proliferation of lymphocytes in mice [4], however we did not find any reports describing antibody production relating to *C. salsa* and *C. deserticola*. Therefore, we tried to investigate the new effects of *C. salsa* on antibody production.

The production of antibodies occurs during one of the latter stages of humoral immunity. Antigen-specific antibody production occurs *via* cell-cell interaction and cytokine signal transduction [5]. Many studies have described B cell activation [6] and interleukin-6 (IL-6) has been shown to be a cytokine that is related to activation of the B cell [7, 8].

Materials and methods

Preparation of the *Cistanche salsa* extract

Dried *Cistanche salsa* (1 g) (produced in China and supplied by Karutan Co., Japan) was cut into small pieces and treated with 250 ml of purified water at 50°C for 30 min. The acquired extract was centrifuged at 13,400×G for 20 min. The supernatant was filtered using a sterilized gauze and then passed through a filter (0.22 μm). The resulting extract was used for the following experiments.

Preparation of human lymph node lymphocytes (LNL)

Lymphocytes from lymph nodes were obtained from lung cancer patients. Lymph nodes were cut

into small pieces and then squeezed between two glass slides [9]. The separated lymphocytes were washed twice with ERDF medium (Kyokuto Pharmaceutical Industrial CO., LTD., Japan). The obtained lymphocytes were treated with 0.25 mM L-leucyl leucine methyl ester (Boehringer GmBH, Germany) to remove the cytotoxic T cells, $CD8^+$ T cells, and natural killer cells before use [10]. The shift from cell-mediated immunity toward humoral immunity is expected to occur by this treatment.

Cell culture

Lymph node lymphocytes were cultured in ERDF medium containing 10% fetal bovine serum (FBS; HyClone Laboratory, USA), 100 U/ml interleukin-2 (Chemicon International Inc., USA) [10] and 50 μM 2-mercaptoethanol [11]. Cells were plated in duplicate at 3.3×10^5 cells/ml under humidified 5% CO_2/95% air atmosphere at 37°C. Cells were harvested by gently using a pipette, leaving the adherent cells. The acquired cells were counted using a hematometer. Adherent cells were not considered when determining the number of cells. Cell death was determined by trypan blue dye exclusion. Viable cell density was calculated by determining the viability and number of cells harvested.

Enzyme-linked immunosorbent assay (ELISA)

The production of IgM and IgG was measured by ELISA. Briefly, microtiter plates were coated with goat anti-human IgM or IgG (BioSource International, Inc., USA) in 50 mM carbonate buffer (pH 9.6) and incubated at 37°C for 1 h. The wells were then washed with phosphate buffered saline (PBS) containing 0.05% Tween20 (TPBS) and the wells were filled with a blocking solution (PBS containing 1% 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 h. The wells were then washing with TPBS, horseradish peroxidase (HRP)-conjugated goat antibody to human IgM or IgG (BioSource International, Inc., USA) was added and the plate was incubated at 37°C for 1h. Finally, the wells were washed three times with TPBS and treated with a substrate solution containing 0.3 mg/ml 2,2'-azino-bis(3-ethylbenzthiazolin-6-sulfonic acid) diluted in 0.1 M

citrate buffer (pH 4.0) containing 0.03% H_2O_2. Absorbance of the color reaction was measured at 405 nm. Antibody productivity was calculated by measuring the amount of antibody produced by a certain amount of cells during a fixed time period.

IL-6 production was similarly measured by ELISA. Briefly, microtiter plates were coated with rabbit anti-goat IgG (Zymed Labotatories, Inc. USA) in PBS and was incubated at 37°C for 1h. After washing wells with TPBS, polyclonal goat anti-human IL-6 antibody (IgG) (R&D System, Inc., USA) was added and was incubated at 37°C for 1 h. Cultured supernatants and standard solution was added and incubated. After the washing with TPBS, biotinylated mouse anti-human IL-6 antibody (Former Genzyme-Techne, USA) was added and incubated at 37°C for 1 h. HRP-conjugated streptavidin (Amersham Bioscience Corp, USA) was added and incubated. After the washing the wells were treated with substrate solution as described above.

Flow cytometric analysis

Cultured cells were washed with PBS containing 10% FBS, and were stained on ice with the appropriate antibody for 30 min. The cells were washed and re-suspended with PBS. Relative fluorescence intensity was detected using a flow cytometer. Antibodies used were as follows: mouse anti-human interleukin-6 receptor (IL-6R) antibody (IgG) (Former Bender Medsystems. AUT); R-phycoerithrin-conjugated goat anti-mouse IgG (H+L) (Immunotech Corp. FRA); FITC-conjugated goat anti-human immunoglobulins (BioSource International, Inc., USA).

Results and discussion

Figure 1 shows the concentration of antibodies produced from LNL treated with various concentrations of the plant extract for 7 days. The extract enhanced both IgM and IgG production dose-dependently. At 0.1%, it was shown to be able to enhance IgM production. Likewise, the extract at 1% demonstrated a similar enhancement of IgG production. At 10%, it was able to increase the production of both IgM and IgG 5-fold.

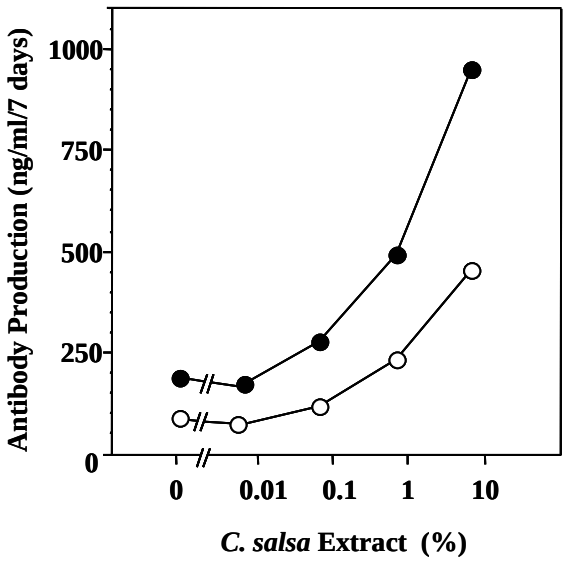

Figure 1. Effect of *C. salsa* extract on antibody production in LNL. ●, IgM; ○, IgG.

Figure 2 shows the viable cell density in non-treated and *C. salsa* extract (3%)-treated cells. Under the microscope, no difference was found in any of the adherent cells. In contrast, antibody productivity was enhanced (Fig. 3). Results show that after 5 days, *C. salsa* extract-treated cells began producing both IgM and IgG greater than non-treated cells.

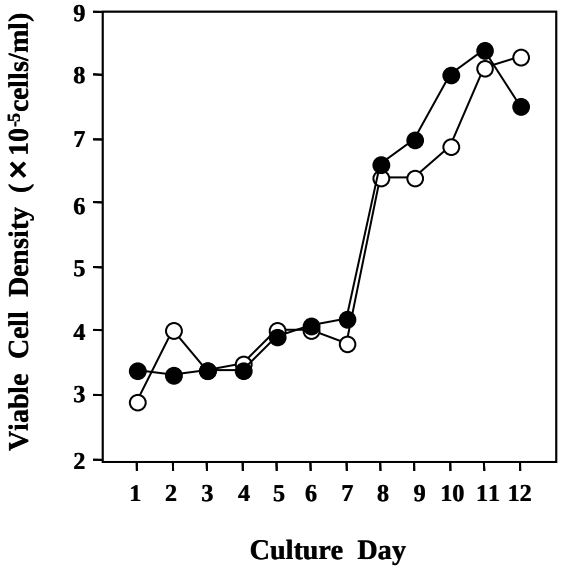

Figure 2. Effect of *C. salsa* extract on the cell growth of LNL. ○, non-treated ; ●, C. salsa extract (3%)-treated.

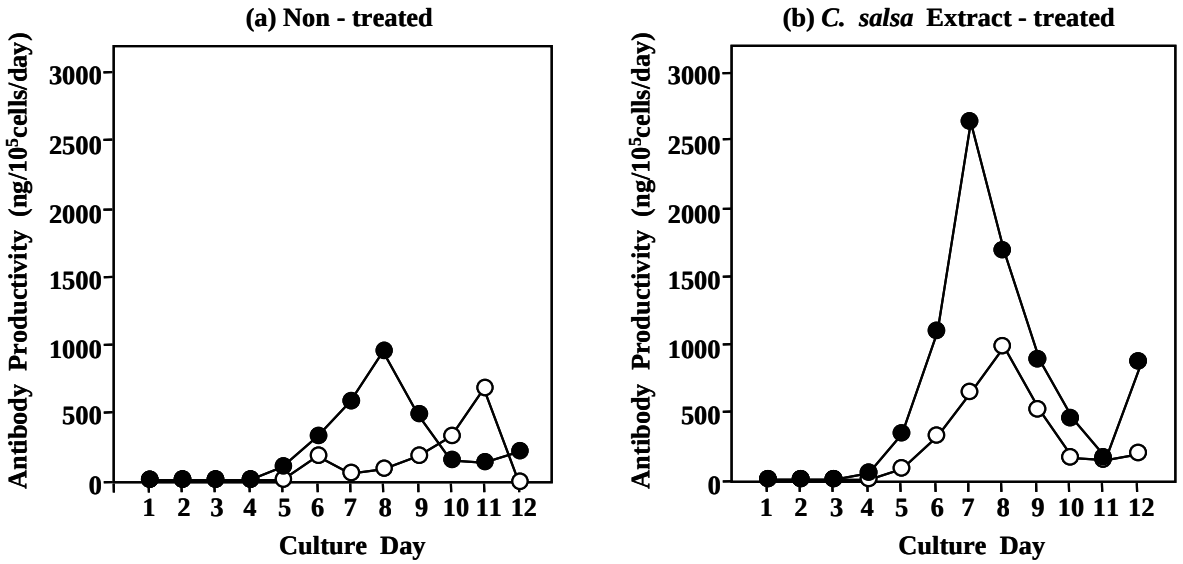

Figure 3. Effect of *C. salsa* extract on IgM and IgG productivity in LNL during a 12 day period. ●, IgM; ○, IgG.

In the immune system, arabinogalactan from *C. deserticola* was shown to induce lymphocyte proliferation after a 3 days treatment [4]. In this study, it seemed that *C. salsa* extract did not induce lymphocyte proliferation. We assumed that the extract was able to activate the non- or low-antibody secreting B cells, however it is still unclear whether or not the extract acts upon the B cells directly. At first we thought the activation was related to IL-6, which is a cytokine involved in B cell activation. However, IL-6 concentration in cultured supernatants was less than the concentration that can be detected by ELISA (data not shown). Burdin et al. reported that germinal center B cells did not secrete IL-6 but the expression of IL-6R was up-regulated [12]. Therefore, we examined IL-6R expression and surface immunoglobulin (sIg) expression on B cells to use as a marker for activation. Results show that IL-6R expression on sIg expressed B cells within LNL was indeed affected by *C. salsa* extract. After culturing with the extract for a 5 day period (day 2 to 7), the expression of IL-6R was increased (Fig. 4). However, the cells treated with the extract for a 7 day time period (day 0 to 7) did not express up-regulated IL-6R (data not shown).

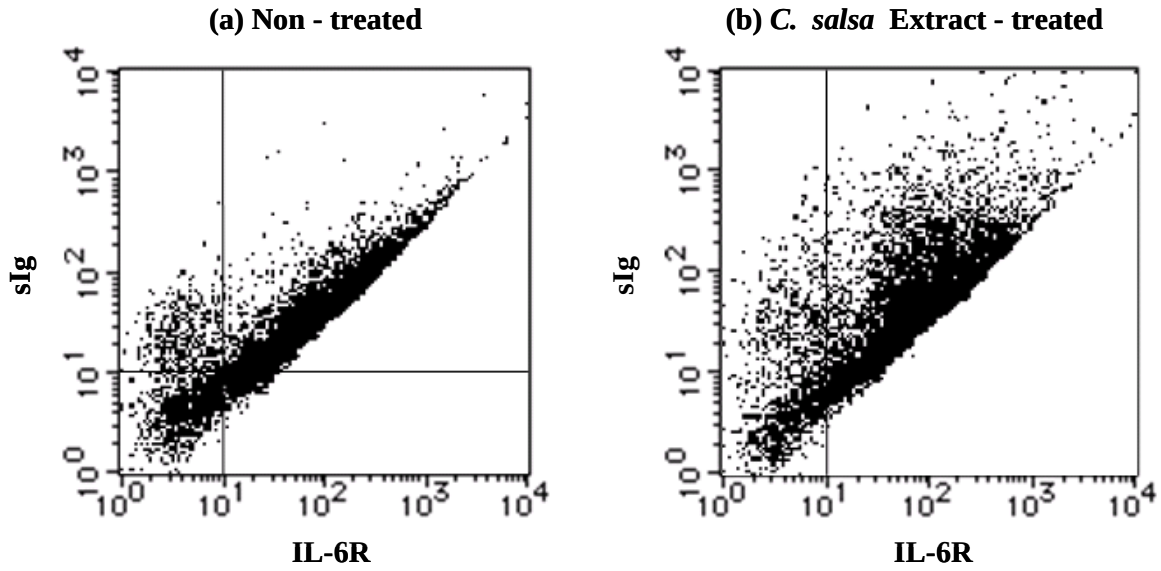

Figure 4. Effect of *C. salsa* extract on IL-6R expression on sIg expressed B cells in LNL.

These results suggest that the *C. salsa* extract-induced activation is not as simple as mitogen-induced activation which is mainly dependent on cell growth. Rather, the induced activation is related to the differentiation accompanying with the up and down regulation of the cytokine receptors.

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