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Maqui Berry Extract Activates Dendritic Cells Maturation by Increasing the Levels of Co-stimulatory Molecules and IL-12 Production

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Abstract

Dendritic cells play a very important role in the immune response as antigen-presenting cells that are critical for initiating both innate and acquired immunity. They recognize, process and present foreign antigens to other key immune cells to trigger and regulate the immune response. The ability to activate these dendritic cells can be used as a treatment for various immune diseases. maqui berry has been reported to have anticancer, antibacterial and anti-inflammatory properties. However, its effect on the activity of dendritic cells has not been studied. In this study, we investigated the efficacy of maqui berry extract in modulating dendritic cell activity. Treatment of dendritic cells with maqui berry extract induced the costimulatory molecules CD80, CD86, and MHC class I and II in a concentration-dependent manner. Furthermore, the antigen-presenting capacity of dendritic cells was inhibited, which confirms their ability to present antigens, and the production of Interleukin (IL)-12, which is important for dendritic cell activity, was increased. These results indicated that maqui berry extract activates dendritic cells maturation by inducing the production of co-stimulatory molecules and IL-12. These results suggest that maqui berry extract may act as an effective adjuvant to enhance dendritic cell-based immune responses.

Keywords: CBP7, Development, *Dictyostelium* Calcium

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1. Introduction

Dendritic cells play a central role in immune responses. The immune responses are mediated by both innate and adaptive immune responses, which involve the intimate interaction of several immune cells, including T cells, B cells, dendritic cells, and macrophages. Among these cells are potent

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antigen-presenting cells that have a profound impact on immune responses, including initiating immune responses, presenting antigens, secreting chemokines, and activating immune cells through their involvement in adaptive immune responses. After recognizing the antigen, dendritic cells must be activated into mature dendritic cells in order to induce an acquired immune response with antigen specificity. In this process, co-stimulatory molecules such as CD80, CD86, MHC class I, and MHC class II are expressed, and antigens are presented to other immune cells to induce an acquired immune response. As dendritic cells play such an important role in the immune system, many researchers are seeking to develop immune adjuvants that can activate dendritic cells in order to regulate them. Finding new immune adjuvants that can increase dendritic cell activity could provide a basic treatment for a variety of dendritic cell-based immune-related diseases.

Maqui berry (Aristotelia chilensis) is endemic to Argentina and Chile. maqui berry contains Argentina and delphinidin, which has been shown to have hypoglycemic and antioxidant effects, and has been associated with metabolic disease inhibition, anti-cancer, anti-microbial, anti-inflammatory, memory enhancement, and inflammatory diseases. However, the direct effects of maqui berry on dendritic cell maturation and activation have not been studied. Therefore, this study was conducted to determine whether maqui berry could directly affect dendritic cell activation.

2. Materials and methods

2.1. Animals

C57BL/6 (8 weeks) mice were purchased from Zibio (Gwangju, South Korea). All animals were housed in a specific pathogen-free (SPF) environment with a 12-hour light/dark cycle.

2.2. Cell viability (Annexin/PI assay)

Annexin/PI experiments were performed using the Annexin V FITC Apop Dtec Kit I (BD Pharmingen™, USA). After cells were washed twice with cold PBS, 10^5 cells/ml cells were added to 100 μl of 1X Annexin V Binding buffer (1X Annexin V Binding buffer containing 5 μl FITC Annexin V and 5 μl PI) and mixed gently. Incubate the sample for 15 min at RT in the dark, then add 400 μl of 1X Annexin V Binding buffer and analyze using a flow cytometer.

2.3. Dendritic cells isolation and culture

The dendritic cell isolation was performed on a sterile workbench and isolated from the bone marrow of the mouse (Bone marrow-derived dendritic cells). The bone marrow obtained by isolating the femurs of the mice was made into bone marrow cells using a syringe. Bone marrow cells are washed to remove red blood cells using Red Blood Cells lysis buffer (Sigma-Aldrich, USA) and cultured at 10^6 cells/ml using complete media (RPMI-1640 supplemented with 10% FBS, 20 ng/ml recombinant GM-CSF and recombinant IL-4). Complete media is changed every 2 days and after 6 days, dendritic cells are collected and used for

experiments using bead-conjugated anti-CD11c mAb followed by positive selection through paramagnetic columns.

2.4. Antigen uptake capacity

Dendritic cells are treated with maqui berry extract at 100 μg/ml, followed by fluorescein (FITC)-conjugated Dextran at a concentration of 1 mg/ml and pulsed at 4°C (negative control) and 37°C for 45 min. The dendritic cells are then washed with PBS, stained with PE-conjugated anti-CD11c and analyzed using a flow cytometer.

2.5. Cytokines assay

Dendritic cells were fixed and permeabilized with the Cytofix/Cytoperm kit (BD Pharmingen) according to manufacturer's instructions. Intracellular IL-12 p40/p70 and IL-10 were stained with fluorescein R-phycoerythrin (PE)-conjugated antibodies (PharMingen) in a permeation buffer. The cells were analyzed using FC500 flow cytometer with the FlowJo program.

3. Results

3.1. Maqui berry extracts induces dendritic cells maturation

We first treated dendritic cells with maqui berry extract at concentrations of 25, 50, and 100 μg/ml for 24 hours to determine if the extract was toxic to the cells before proceeding with further experiments (**Fig. 1.**). The results showed no cytotoxicity from the maqui berry extract. For dendritic cells to mount an effective immune response to foreign antigens, they must mature and express co-stimulatory molecules such as CD80, CD86, MHC class I, and MHC class II.

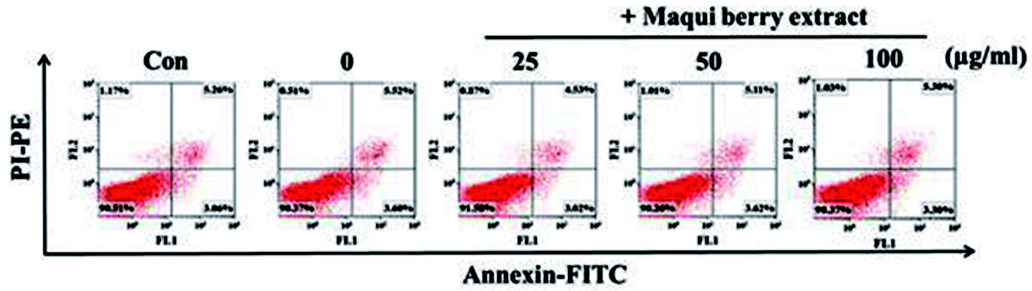

Fig. 1. Maqui berry extract had no cytotoxic effect on dendritic cells. Dendritic cells were incubated with maqui berry extract at a concentration from 25 to 100 mg/ml for 24 h. The cell viability was determined by Annexin-V/PI staining and the results are expressed as the percentage of positive or negative cells.

Therefore, our aim was to determine if maqui berry extract would induce dendritic cell maturation and enhance the expression of co-stimulatory molecules. We treated dendritic cells with maqui berry extract at concentrations of 25, 50, and 100 μg/ml, using LPS as a positive control, for 24 hours to measure the expression of CD80, CD86, MHC class I, and MHC class II, which are markers of dendritic cell maturation. As shown in **Fig. 2.**, we found that maqui berry extract increased the expression of

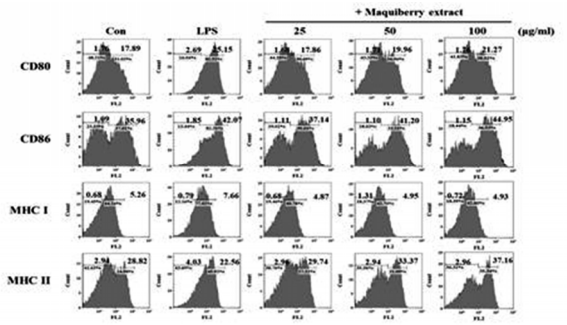

Fig. 2. Maqui berry extract induces co-stimulatory molecules on dendritic cells. Dendritic cells were treated with CON (medium only), LPS (200 ng/ml) or maqui berry extract (25, 50, 100 mg/ml) for 24 h and the cells were collected and stained with fluorescence-conjugated specific antibodies (CD80, CD86, MHC class I and II). Stained cells were analyzed with flow cytometry.

maturation markers on dendritic cells in a dose-dependent manner. Therefore, we confirmed that maqui berry extract affects dendritic cell maturation and may play a role in the regulation of dendritic cell-based immunity.

3.2. Maqui berry extract modulates immune response by inducing a decrease in antigen uptake capacity

In general, mature dendritic cells are known to be involved in the immune response with reduced antigen uptake capacity and enhanced expression of motility-related chemokines compared to immature dendritic cells. We have shown in previous experiments that maqui berry extract induces the maturation of dendritic cells. Therefore, to determine how maqui berry extract affects dendritic cell antigen uptake, which is known to be a fundamental function of dendritic cells, we assessed dendritic cell antigen uptake capacity. As shown in **Fig. 3.**, we found that the

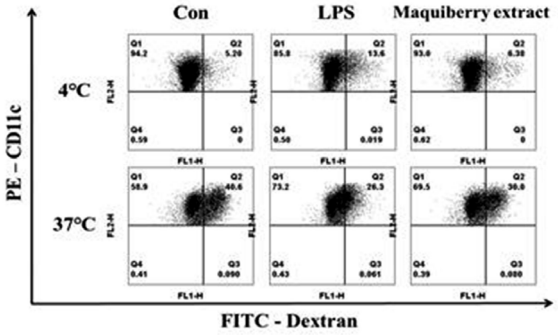

Fig. 3. Maqui berry extract-treated dendritic cells regulate endocytic capacity. Dendritic cells were treated with 100 mg/ml of maqui berry extract and LPS (200 ng/ml) for 24 h. The antigen uptake capacity was determined by flow cytometry after dextran-FITC incubation. Cells were washed with cold PBS and stained with PE-conjugated anti-CD11c antibody. The antigen uptake capacity of the controls was determined after exposure to dextran-FITC at 4 °C.

CD11c-PE^+ dextran-$FITC^+$ fraction, which indicates antigen uptake, is lower in the maqui berry extract treatment group than in the normal control group. We repeated the same experiment at 4°C and found that antigen uptake by dendritic cells was inhibited at low temperatures. These results suggest that maqui berry extract not only induces the maturation of dendritic cells but also affects their function.

3.3. Maqui berry extract induces IL-2 production in dendritic cells

IL-12 secreted from dendritic cells is a representative cytokine that increases the activity and proliferation of T cells and induces the Th1 immune response. Furthermore, IL-12 has been reported to increase cellular immune responses against tumor cells by increasing the secretion of IFN-γ, which induces Th1 immune responses. Therefore, in this study, we tried to

determine whether the maqui berry extract increases the production of IL-12. As a result of confirming the production of IL-10 and IL-12 by treating dendritic cells with 100 μg/ml of the maqui berry extract, it was confirmed that the production of IL-12 was increased as compared with the control group (**Fig. 4.**).

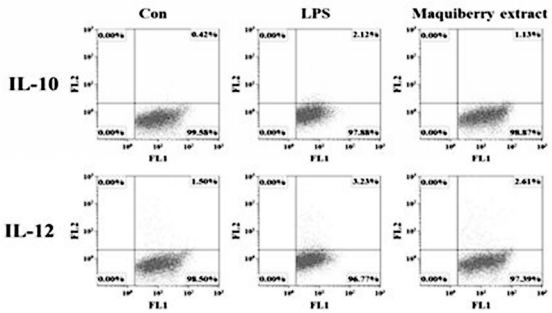

Fig. 4. Maqui berry extract induces IL-12 production of dendritic cells. Dendritic cells were treated with con (medium only), LPS (200 ng/ml) or maqui berry extract (100 mg/ml) for 24 h. Represents the analysis of IL-12 and IL-10 production in dendritic cells by intracellular cytokine staining after 24 h of maqui berry extract stimulation.

4. Discussion

Dendritic cells are potent antigen-presenting cells that can initiate both innate and adaptive immunity, and their maturation and activation are important parts of the immune response. Therefore, many researchers are continuously studying the development of new immunologic al adjuvants that can activate dendritic cells and identify their functions. For this reason, we hypothesized that maqui berry extract, which has antioxidant, anti-inflammatory, and anti-cancer properties, might be able to activate dendritic cells. The results showed that treatment of dendritic cells with maqui berry extract did not show cytotoxicity, but increased the expression of co-stimulatory factors involved in dendritic cell maturation and activation, such as CD80, CD86, MHC class I and II, indicating that maqui berry ext ract induces dendritic cell maturation (**Fig. 2.**).

Additionally, the antigen uptake capacity was investigated, and it was found that maqui berry extract induced dendritic cell maturation. The production of IL-12, a cytokine important for dendritic cell activity and T cell activity, was also determined and found to be increased by maqui berry extract treatment compared to the control (**Fig. 4.**). Taken together, these results suggest that maqui berry extract has dendritic cell activating effects by inducing the production of dendritic cell co-stimulatory factors and IL-12. Therefore, we suggest that maqui berry extract has the potential to be used as a therapeutic agent for various immune diseases based on dendritic cells.

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