Introduction

Eckol is a purified compound of Ecklonia cava, a brown alga that is a foodstuff in Korea. It has been demonstrated that eckol is able to function as an antioxidant (1) and inhibit mushroom-derived tyrosinase activity (2). Especially, recent study reported that eckol protects cells against hydrogen peroxide, serum starvation, radiation-induced cell death and increases the activity of catalase, the phosphorylation of extracellular signal-regulated kinase (ERK), and the activity of NF-κB transcription factor (3).

Dendritic cells (DCs) are the most potent antigen-presenting cells that play critical roles in immune systems of host, such as inducing immune tolerance, both innate and adaptive immune responses (4-7).

Materials and Methods

**Animals and reagents.** C57BL/6, BALB/c mice were purchased from ORIENT BIO, Inc. (Seongnam, Gyeonggi, Korea) and maintained in the lab animal facility. 7- to 12-week-old female mice were used for experiments. Animal experiments were performed based on the NIH guidelines (USA) for laboratory
animal use and care. Eckol was obtained from Dr. Nam Ho Lee (Cheju National University, Jeju, Korea) and a previous report provided its structure (3). Anti-CD40 mAb (BD Biosciences, San Jose, CA, USA) was used for CD40 ligation, a physiological maturing signal for DCs. In this study, anti-CD40 mAb was used as a positive control for maturation in most experiments except for the determination of nitric oxide (NO) since it did not induce the production of NO in preliminary experiments.

Generation of DCs. DCs were generated from bone marrow cells of C57BL/6 mice using a protocol that was established in our lab (8). In brief, bone marrow cells were harvested from femur and tibia of mice by flushing with Hanks’ balanced salt solution (HBSS, Invitrogen, Carlsbad, CA, USA) and erythrocytes were removed using ammonium chloride, potassium carbonate (ACK) lysis buffer. Cells were cultured at a concentration of 2×10⁶ cells/ml in 6-well culture plates. RPMI-1640 medium containing 2 mM L-glutamine, 5% fetal bovine serum (FBS), 100 U/ml penicillin/streptomycin (all from Invitrogen) and 10 ng/ml recombinant mouse granulocyte-macrophage colony-stimulating factor (GM-CSF, Biosource International, Camarillo, CA, USA) was used for DC culture. At 6 to 10 day of culture, floating cells, over 85% CD11c⁺ cells based on flow cytometric analysis, were used as DCs for experiments.

Measurement of cell viability. For the determination of cell viability, we performed 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, St. Louis, MO, USA) assay. After staining, cells were analyzed with FACSCalibur™ flow cytometer and CellQuest™ software (BD Biosciences). Splenic CD4⁺ T lymphocyte preparation and mixed lymphocyte reactions (MLR). Spleen cells were harvested from spleen of Balb/c mice using a protocol that was previously established (9). For MLR, CD4⁺ T lymphocytes were purified from the spleen cells using CD4 (L3T4) MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer’s instructions. DCs of C57BL/6 and 1×10⁵ CD4⁺ T cells of Balb/c were co-cultured in 96-well flat-bottom cell culture plates (BD Biosciences) and the ratio of DC: T cell was a range of 1 : 20 ~ 1 : 540 in quadruplicate. The cocultures were incubated for 5 days and pulsed with ¹H-thymidine (PerkinElmer, Wellesley, MA, USA) at a concentration of 1µCi/well for last 18 hr. Incorporated thymidine was measured by a scintillation counter, Wallac Microbeta® TriLux (PerkinElmer).

Effect of eckol on the viability of DCs. To investigate the effects of eckol on DCs, we first measured the viability of DCs treated with eckol. In MTT assay, eckol marginally increased the viability of DCs in a concentration-dependent manner (Fig. 1). After culture of DCs in the presence of GM-CSF, DCs were used for experiments in the absence of GM-CSF. This leads to cytokine withdrawal-induced apoptosis of DCs. It is thus suggested that eckol may protect DCs from the apoptosis. To eliminate the possibility that eckol dissolved in DMSO may enhance the O.D. value by chemical reaction with MTT reagent, we subtracted the O.D. value of eckol itself from that of eckol-treated DCs. It is thus suggested that eckol may protect DCs from cytokine withdrawal-induced apoptosis.

Eckol does not affect the production of NO or IL-12 from DCs. To investigate if eckol may have a potential character of inflammatory agent, we measured the level of NO from eckol-treated DCs. Eckol did not enhance the
significant level of NO at a range of concentration (0~33 μg/ml) and only marginal increase at 33 μg/ml (Fig. 2A). In same experiments, LPS, as a representative pathological maturing agent for DCs, markedly increased the level of NO. In addition, we measured the level of IL-12, a major inducing cytokine for helper T cell type 1 (Th1) or cellular immunity. The level of IL-12 produced by eckol-treated DCs was very similar to that by imDCs (Fig. 2B).

**The enhanced expression of maturation markers on DCs by eckol.** To investigate if eckol may affect the maturation status of DCs, phenotypic analysis was performed using flow cytometric staining. MHC class II and CD86 molecules have been recognized as maturation markers for mouse dendritic cells originated from bone marrow cells. Flow cytometric analysis revealed that eckol-treated DCs expressed more MHC class II and CD86 molecules on the surface compared to imDCs (Fig. 3). Anti-CD40 mAb-treated DCs, as a positive control for mature DCs, expressed the highest levels of those molecules. It is thus suggested that eckol may convert viable DCs into a certain status of mature DCs.

**The increased alloproliferation of CD4+ T lymphocyte activated eckol-treated DCs.** As a crucial role in immune system, DCs have the capability to initiate the activation of naïve T lymphocytes. We measured the stimulatory ability of eckol-treated DCs for alloproliferation, which was quantified by 3H-thymidine incorporation (Fig. 4). Eckol-treated DCs significantly enhanced the alloproliferation at a ratio of DC (5×10^5 cells/well) and CD4+ T lymphocytes (1×10^5 cells/well). Anti-CD40 mAb-treated DCs, as a positive control for mature DCs, markedly stimulated the alloproliferation of CD4+ T lymphocytes. It is suggested that eckol may enhance the stimulatory capability of DCs for allo-
stimulation, which is recognized a role of mature DCs.

IL-4 production was enhanced from CD4⁺ T lymphocytes by eckol-treated DCs. In the co-culture of CD4⁺ T lymphocytes and DCs, we measured the levels of cytokines (Fig. 5), IFN-gamma and IL-4, a representative cytokine of Th1 and Th2 respectively. In the interest of accuracy, we performed the quantification of both IFN-gamma and IL-4 in same samples. Eckol-treated DCs significantly enhanced the production of IL-4 from CD4⁺ T lymphocytes compared to imDCs. And also, the level of IFN-gamma was marginally increased in same samples.

Discussion

Eckol is a pure compound originated from one of algae, Ecklonia cava. It has been studied for many years and elucidated to have its anti-oxidative (1,3) and inhibitory activity for enzymes, which are critical for a certain condition or pathogenic virus (2,10). Most studies have been focused on established cell lines, but primary cultured cells were used in our study.

It was demonstrated in our study that eckol-treated DCs increased the maturation markers on the surface of DCs, but did not enhance the production of IL-12 compared to imDCs (medium alone-treated DCs). Thus, we do not exclude the possibility which eckol-treated DCs may generate some anergic CD4⁺ T lymphocytes. Based on our data, it is likely that eckol-treated DCs may stimulate CD4⁺ T lymphocytes via cell-to-cell contact.

The eckol-treated DCs significantly enhanced the proliferation of allogeneic CD4⁺ T lymphocytes compared to imDCs. And also, the activated CD4⁺ T lymphocytes by eckol-treated DCs produced more IFN-γ and IL-4 compared to those cells by imDCs. These data suggest that eckol may at least have some maturing effects on DCs. Although we tried to investigate Th1 or Th2 shift of CD4⁺ T lymphocytes activated by eckol-treated DCs, we observed a similar pattern of the production of two cytokines to those cells by anti-CD40 mAb-treated DCs from ELISA assay. It was suggested that the difference of cytokine amount from activated CD4⁺ T lymphocytes by eckol-treated DCs and anti-CD40 mAb-treated DCs might be attributed from the proliferation rate of activated CD4⁺ T lymphocytes (Fig. 4). The effects of eckol on immune system in vivo and specific immune cells in diseases or pathologic conditions were needed to be studied in future.

Taken together, we demonstrated in this study that eckol modulated immune responses via changing the phenotype and function of DCs. The effects of eckol

![Figure 4](image-url)  
**Figure 4.** Eckol-treated DCs increased the alloproliferation of CD4⁺ T lymphocytes. DCs were harvested and treated as Fig. 2B. And then, DCs were washed with HBSS twice and co-cultured with allogeneic CD4⁺ T lymphocytes in 96-well culture plates. Incorporated 'H-thymidine in cells was measured as counts per minute (cpm) by a scintillation counter. Data represent mean ±SD of cpm and one of three independent similar results. **indicate p<0.01.

![Figure 5](image-url)  
**Figure 5.** IL-4 production of CD4⁺ T lymphocytes was enhanced by eckol-treated DCs. In same experiments as Fig. 4, the supernatants were harvested from the co-culture of 5×10⁶ cells/well of DCs and 1×10⁵ cells/well of CD4⁺ T lymphocytes, and used for the determination of IFN-γ (A) and IL-4 (B) using ELISA. Data represent mean±SD and one of two independent similar results.
on immune cells were first studied in our knowledge. Especially, since DCs are very specialized antigen-presenting cells, playing a critical role in cellular and humoral immune systems, our study may provide the information about eckol with new aspects for further use.

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