INTRODUCTION

Echinacea is a popular herb used widely in the world for the treatment of common cold and its side effects. It remains one of the most commonly used "dietary supplement" in US (Figure 1) with a reported annual sales more than $300 million (1). It is deployed against wide ranging pathologies but most commonly upper respiratory tract infections (URIs). Due to legends and literatures, the beneficial effect of Echinacea comes from its boosting of immune system via stimulations of antigen-presenting cells (APC), natural killer cells (NKC) and dendritic cells (DC) and improved neutrophil phagocytosis (2,3). Recent clinical study, however, showed that Echinacea purpurea was not effective in treatment of upper respiratory tract infection in children 2 to 11 years old (4). Use of Echinacea associated with an increased risk of irritation was a conclusion. The benefit and detriment of taking Echinacea are therefore to be continuously concerned by the public. In this communication, we intend to understand the influence of Echinacea on humoral-immune effect via the activation of T-cell.

MATERIALS AND METHODS

Reagents

Anti-human CD28 antibody and anti-human CD3 antibody were purchased from BioLegend, FK-506 and ionomycin were purchased from CalBiochem and both of them were dissolved in DMSO at concentration of 1 g/ml. Phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma. PMA was dissolved in DMSO and stored at -20°C. Human CD28-muIg and CD80-muIg fusion proteins were purchased from ID Labs Inc. CD28-muIg is a soluble fusion protein consisting of the extracellular (134 aa) domain of human CD28 fused to murine IgG2a Fc (233 aa). CD80-muIg is a soluble fusion protein consisting of the extracellular (173 aa) domain of human CD80 fused to murine IgG2a Fc (232 aa). Both were purified from tissue culture supernatant of CHO transfectants by protein A and size exclusion chromatography.

Preparation of Crude Extracts of Echinacea (CEE)

50 g sample of Echinacea powder (Echinacea purpurea, SaveOn-Albertson’s™ Inc., USA) was extracted with 100% dd-H₂O (1000 ml) by stirring at room temperature for 72 hours. The maceration extracts were collected and filtered through the filter-paper (No.1, code no. PW300-1125, pore size=10 m, TOYO Inc.). Extraction was repeated for three times. All filtrates were collected and concentrated to 500 ml under reduced pressure. 4000 ml acetone was added to the concentrate and the solution was stocked at -20°C for 24 hours. Afterward, the solution was centrifuged at 8000 rpm for 60 min. The supernatant was collected and then completely dried under reduced pressure.

Surface Plasmon Resonance (SPR)

BIAcore 3000 (Biacore, Uppsala, Sweden) was employed for real-time biospecific
interaction analysis. Binding analysis was performed at 25°C with a flow rate of 10 µl/min. In general, proteins can be immobilized on the layer of carboxylated dextran in CM5 sensor chip (research grade; Biacore) by amine coupling. With each injection, flow-cell can be regenerated to a final concentration of 15 to 30 µM NaCl, 3.4 mM EDTA, 0.005% surfactant P20) buffer HBS (10 mM HEPES, pH 7.4, 150 mM NaCl, containing 50 mM NaOH. Extracts were by flowing though the cell with the buffer each injection, samples were diluted in the running

Biacore) by amine coupling. With each injection, flow-cell can be regenerated to a final concentration of 15 to 30 µM NaCl, 3.4 mM EDTA, 0.005% surfactant P20) buffer HBS (10 mM HEPES, pH 7.4, 150 mM NaCl, containing 50 mM NaOH. Extracts were by flowing though the cell with the buffer each injection, samples were diluted in the running

Bioassay

Jurkat leukemic T cells were maintained in a humified atmosphere of 5% CO₂/95% air at 37°C in RPMI-1640 medium (HyClone, Utah, USA) including penicillin, streptomycin and 10% heat-inactivated FBS. Two modes of activations were used for the present experiments: anti-CD3 plus anti-CD28 (CD28-dependent stimulation) and PMA plus ionomycin (CD28-independent stimulation). For experiment of CD28-dependent stimulation, flat-bottom 96-well plates were coated with 10 µg/ml of anti-CD3 for 24 h at 4°C. Wells anti-CD3 were washed twice with PBS. After washing, the unbouded anti-CD3 was removed. Jurkat T-cells (200 µl, 2×10⁶ cells/ml) were added into the wells. The cells were activated by anti-CD3 already existing in the wells. Upon the

activation, the cell solution was stayed for 24 h. The supernatant (100 µl) of cell solution was then used for the measurement of IL-2 by enzyme-linked immuno-sorbent assay (ELISA). For experiment of CD28-independent stimulation, 200 µl Jurkat cell (5×10⁵ cells/ml) were incubated with PMA (50 nM)/ionomycin (1 g/ml) for 24 h. 100 µl supernatant was then used for IL-2 measurement by ELISA. For experiment of CEE on T-cell, these materials were added into the culture plates including the cells and pre-incubated for 15 min before stimulation. Afterwards, the cells including CEE were stimulated via either CD28-dependant or CD28-independent stimulations. The culture supernatants in both modes were harvested after 24 h incubation and then used for the measurements of IL-2. IL-2 measurement was done using the commerical available ELISA kit (BioSource; California, USA).

RESULTS AND DISCUSSION

CCE was performed in vitro for the investigation on the inhibition of T-cell. During T-cell activation, the interleukin-2 (IL-2) expression (2) of CD28-dependent and CD28-independent pathways in the Jurkat T-cell culture line were conducted with or without the addition of CEE. As an illustration, Figure 2 shows Jurkat IL-2 secretion (%), in response to a CD28 dependent anti-CD3 and anti-CD28 signaling profile (white column, IL-2 secretion via “activation” is normalized as 100%), significantly reduced with application of CEE (white column, ~34%) or, as a reference, the clinical immuno-suppressant FK506 (white and gray columns, ~3%). However, no reduction is observed for CEE (gray column, ~99%) in CD28-independent ionomycin and PMA signaling IL-2 secretion. These outcomes imply that Echinacea’s inhibition is through CD28 dependent pathway. Figure 3 shows sensorgrams of surface plasma resonance (SPR; Biorec 3000) for CD80 bound to CD28 immobilized on a chip without pre-binding of CEE (curve in black) and with pre-binding of CEE (curve in gray). SPR response unit has been largely reduced if pre-binding of CEE was conducted. This implies that certain compounds of CEE can compete the binding with CD80 to block the signal used for producing the IL-2 in T-cell. Related results reported by Philip Huxley et al. (3) showed that synthetic small compounds can block the binding between CD28 and CD80 that inhibit the activation of T-cell. Our results further imply that many herbal medicines might generally practice this “blocking” action in cells including immune cells. For public health, toxicity induced in part from these herbal medicines might therefore be concerned.

It is possible that both enhanced (e.g., to boost the immune system by stimulating antigen-presenting cells, natural killer cells, dendritic cells and by enhancing neutrophil phagocytosis (4,5)) and adverse (e.g. to suppress T-cells) effects are cooperated and balanced in certain extent at different immune cells. Which one is dominated, that seems difficult to be answered so far. Since T-cells play vital role in the immune system, therefore, we believe that the adverse effect (immuno-suppressive effect) of Echinacea founded here could be significant in the immune system.

REFERENCES

2) Li L., Yee C., Bravo J.A. Science 1999, 283, 848-51