Journal of Medicinal Plants Research Vol. 6(3), pp. 534-543, 23 January, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1554
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

The anti-leukemic lectins from *Canavalia ensiformis* induce macrophage differentiation through cross-regulation between monocytes and lymphocytes

Yu-Yawn Chen[1], Chii-Ming Jiang[2], Shih-Hao Wang[2], Ming-Kuei Shih[3], Hsuan-Yun Hu[4] and Hui-Fen Liao[4]*

[1]Department and Graduate School of Physical Education, National Taiwan Sport University, Taichung, Taiwan.
[2]Department of Seafood Science, National Kaohsiung Marine University, Kaohsiung, Taiwan.
[3]Department of Food and Beverage Management, National Kaohsiung Hospitality College, Kaohsiung, Taiwan.
[4]Department of Biochemical Science and Technology, National Chiayi University, Chiayi, Taiwan.

Accepted 6 December, 2011

Lectins from *Canavalia ensiformis* (CEL) have agglutinin effect and anti-leukemic function, however, the immunomodulatory activity of CEL in regulating immune-network remains unclear. The present study clarified CEL effect on modulating the cross-reaction between monocytes and lymphocytes. The hemagglutination activity of CEL was heat-inactive and CEL at low concentrations showed no cytotoxicity to human leukemic U937 cells. However, CEL stimulated mononuclear cells (MNCs), isolated from peripheral blood, to secrete cytokines, such as TNF-α and IL-1β effectively inhibited cell growth and induced monocyte-differentiation of U937 cells. Monocytes contained in CEL-treated MNCs significantly increased the percent of macrophage morphology, formazan-positive cells, and phagocytic activity. The conditioned medium (CM) collected from CEL-treated monocytes (CEL-Mo-CM) markedly increased the viability of both monocytes, while CM from CEL-treated lymphocytes (CEL-Lym-CM) did not have this effect. The activated lymphocytes secreted IFN-γ and then induced macrophage differentiation and CD40 expression through cell-to-cell interaction. Conclusively, CEL directly stimulated monocytes to produced cytokines, which may had autocrine and/or paracrine effects to activate the cross-regulation between monocytes and lymphocytes, result in inducing macrophage differentiation and may cause anti-leukemic immunity against U937 cells.

Key words: Lectins, *Canavalia ensiformis*, macrophage differentiation, anti-leukemic immunity.

INTRODUCTION

Lectins, carbohydrate-binding proteins, are broadly contained in microorganisms, fungi, plants, and animals which specifically recognize glycoproteins (such as glycosylated antigens and markers) in the cell membrane of different tissues, tumors and cellular subpopulations (Martínez-Cruz et al., 2001). For example, rice lectin specifically binds to N-acetyl-glucosamine (GlcNAc) and mannose-containing glycoconjugates (Hirano et al., 2000), lectin from *Amaranthus leucocarpus* recognizes N-acetyl-D-galactosamine (GalNAc) residues and O-glycosidically linked glycan, and wheat-germ agglutinin interacts with sialoglycoconjugates (Monsigny et al., 1980). The glycoconjugate specificity of lectins causes agglutinating activity according to specific erythrocytic blood types, as with lectin from *Zea mays* which possesses anti-B type specificity (Prodanov and Atanasova, 1984).

Because lectins mediate cell-cell interactions by combining with complementary carbohydrates on apposing cells, they play a key role in modulating various processes in living organisms (Sharon and Lis, 1989). Mody et al. (1995) indicated that lectins contribute to tumor cell recognition, cell adhesion and localization, signal transduction across membranes, mitogenic stimulation, host-immune defense augmentation,

*Corresponding author. E-mail: liaohf@seed.net.tw. Tel: +886-5-2717779. Fax: +886-5-2717929

cytotoxicity, and apoptosis regulation. Masnikosa et al. (2010) also reported that human serum protein insulin-like growth factor binding protein (IGFBP)-3, which contains three N-linked glycosylation sites which broadly interact with lectins, may be a potential diagnostic marker for different illnesses. In Burkitt's lymphoma, loss of lectins (L-PHA, PNA, or ConA)-reactive oligosaccharides is closely associated with a poor prognosis in patients (Suzuki et al., 2007). Therefore, development of lectin-based diagnostic and therapeutic tools for cancers and other diseases may be an important strategy (Mody et al., 1995).

Moreover, carbohydrate recognition of lectins has been reported recently as allowing for diverse biological roles. Lectin from mushroom *Agaricus bisporus* (ABL) has been reported to inhibit proliferation of human colorectal and breast carcinoma cells without any toxic effects (Yu et al., 1993). β-glactoside-binding mistletoe lectin (ML)-I (also named *Visum album* agglutinin, VAA), evidenced as being cytotoxic through the inhibition of protein synthesis in cancer cells (Gabius et al., 1994), induced apoptosis in human myelomonocytic leukemic cells (Joshi et al., 1994), and bound to multidrug resistance-associated protein MRP5 in drug resistant cells (Nehmann et al., 2009). Lectins also act as adhesion molecules in the process of metastasis (Mody et al., 1995). Lymphokine-activated lymphocytes (LAK) contain high levels of soybean lectin receptors which interact with E-cadherin in drug resistant breast tumor cells elevating antitumor activity (Berezhnaya et al., 2009).

The most important role of lectins is as a biological response modifier (Mody et al., 1995). The immunomodulatory effects of ML-I, including stimulation of macrophage activity (Beuth et al., 1991), increased the number of T, helper T, and NK cells (Beuth et al., 1992) and induced cytokine production (Boccil, 1993). The increased concentration of plasma protein mannose-binding lectin (MBL), which activates the complement system by binding to carbohydrates thus acting as an important component of the innate immune system, could reduce susceptibility to infection in chemotherapy patients (Peterslund et al., 2001).

The seed and root of *Canavalia ensiformis*, similar to *Canavalia gladiata*, have been used for hundreds of years in traditional Chinese medicine to maintain health. Murakami et al. (2000) isolated several physicochemicals, such as canavalioside and eight gladiatosides, from the seed of *C. gladiata*. A dietary legume 4-O-methylgallic acid contained in *C. gladiata* enabled antiangiogenic activity (Jeon et al., 2005). Lectins from *C. ensiformis* (CEL), especially Concanavalin A (Con A), induced the expression of Toll-like receptors (TLRs) 1-9 in murine peritoneal macrophages (Sodhi et al., 2007), and regulated the production of inflammatory cytokines and nitric oxide (Kesherwani and Sodhi, 2007).

Con A, a kind of mitogen, effectively stimulates proliferation of peripheral blood mononuclear cells (Renner et al., 2011). Additionally, low doses of Con A enhanced innate immune responses and prevented liver injury in mice (Conchon-Costa et al., 2007). However, the immunomodulatory effects of CEL in immunocompetent networks, including macrophage and lymphocyte regulation, were still unclear. To realize the possible regulatory effects of CEL, with and without heat treatment, the present study developed an *in vitro* model and clarified the cross-reaction between monocytes and lymphocytes and induction of macrophage differentiation.

MATERIALS AND METHODS

Agglutination activity

Human type O erythrocytes were isolated from peripheral blood and then treated with serial diluted CEL or heat (100°C, 5 min)-treated CEL (HCEL) at a concentration of 1 μg/ml in a 96-well plate for 2 h. The agglutination activity of red blood cells was observed and photographed.

Human leukemia U937 cell line and mononuclear cells (MNCs), lymphocytes, and monocytes from human peripheral blood

The human monoblastoid leukemic cell line U937 cells, obtained from the American Type Culture Collection (Manassas, VA), were cultured in an RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS, Hyclone, Logan, Utah) and maintained in an exponential growth state used to assay the effect of CEL or HCEL on cell viability and differentiation. Peripheral blood samples were obtained from 12 untreated healthy donors (20 to 30 years old) with approval by IRB and signed informed consents. MNCs from human blood were separated by centrifugation on a density gradient (Ficoll-Hypaque, 1.077 g/ml, Pharmacia Fine Chemicals, USA) and a concentration of 2×10^6 cells/ml was incubated in an RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated FBS. After 2 h of culturing, the MNCs, the non-adherence lymphocytes and the adherence monocytes were collected separately. MNCs, lymphocytes (Lym) and monocytes (Mo) were treated with or without various concentrations of CEL or HCEL at 37°C in a humidified 5% CO_2 incubator for 24 h; the cell-free supernatant was then collected as the conditioned medium (MNC-CM, Lym-CM, and Mo-CM, respectively) and stored at -70°C until required. Phytohemagglutinin (PHA; Difco Lab., Detroit, MI) was used in this study as the positive control. To rule out possible endotoxin contamination, the CEL- or HCEL-stimulated cells were prepared in the presence of 50 μg/ml polymyxin B (Sigma, St. Louis, MO) in the experiments of cell viability and differentiation assay.

Cell viability assay

Cells were incubated in 35 mm Petri-dishes at an initial concentration of 1×10^5 cells/ml in the presence of various concentrations of CEL, HCEL, 30% (v/v) treated MNC-CM, or normal MNC-CM. The numbers of viable cells were counted using the trypan blue dye exclusion test. Adherent cells were collected by gently rubbing the dishes with a rubber policeman (Bellco Glass, Vineland, USA) and counted using the trypan blue dye. Alternately, we examined the cell viability using a tetrazolium dye colorimetric (MTT) assay method and measured specimens

spectrophotometrically at a wavelength of 550 nm.

Maturation profile of monocytes/macrophages

After the various treatments, the cells were collected and cytocentrifuged onto a microscope slide using a Cytospin^2 (Shandon Southern Instrument Inc., Pittsburgh, PA), stained with Wright's stain and observed under an inverted microscope (Olympus, Tokyo, Japan) with a magnification of 1000×. Based on morphology, the cells were classified as immature cells and mature monocytes or macrophages.

Assay for superoxide production

The production of cytoplasmic superoxide by differentiated myeloid cells was detected by the nitroblue tetrazolium (NBT) reduction test (Liao et al., 2006). The treated cells were suspended in a RPMI 1640 medium at a concentration of 1×10^6 cells/ml and incubated for 30 min at 37°C with an equal volume of NBT test stock solution containing 2 mg of NBT and 1 μM of phorbol myristate acetate per ml of phosphate-buffered saline (PBS). Cytospin preparations were counter-stained with 0.5% Safranin O (Sigma). The percentage of formazan-positive cells (out of 200 cells) was assessed microscopically.

Assays for phagocytosis

The phagocytic activity was measured according to the methods used in our previous study (Liao et al., 2006). Briefly, a yeast suspension was prepared by suspending yeast in PBS at a concentration of 1×10^8 cells/ml in stock. The cells were collected, washed, re-suspended (1×10^6 cells/ml) in FBS-containing RPMI1640 medium, and incubated with the yeast suspension (4×10^6 cells/ml) at 37°C for 30 min. Then, the cells were placed on a glass slide and observed under an inverted microscope. The percentage of yeast-containing cells was taken from 200 cells.

Measurement of cytokines

To detect differentiation-inducing factors secreted from MNCs or lymphocytes contained in the conditioned medium, commercial kits (from R&D systems, Minneapolis, MN) for interferon-gamma (IFN-γ), interleukin-1beta (IL-1β), and tumor necrosis factor-alpha (TNF-α) were used to perform enzyme-linked immuno-sorbent assay (ELISA).

Immunofluorescent staining of CD40 expression

The CEL-treated cells were collected and reacted with an anti-CD40 primary antibody (1:1000, Santa Cruz, Heidelberg, Germany) and an immufluorescence PE-conjugated anti-IgG-TR antibody (1:500, Santa Cruz) in order to determine the distribution of CD40 expression in cells. Hoechest 33342 fluorescence dye was also used to stain the location of the nucleus. The cells were then photographed under a fluorescence microscope at a magnification of 400X.

Effects of CD40 blocking peptide on CEL-induced macrophage differentiation

MNCs were treated without and with CEL (10 μg/ml) in the presence or absence of the CD40 blocking peptide (80 ng/ml, Santa Cruz) and co-cultured for 24 h. The monocytes separated from the treated MNCs were collected and assayed in regard to NBT reduction test, as described earlier. Additionally, CD40 blocking peptide was added in CEL-treated MNC and co-cultured for 24 h. Then, CEL-MNC-CM was collected for analysis the inhibitory effect on U937 cells.

Statistical analysis

The results are expressed as the mean ± standard deviation (SD) of at least three experiments. Statistical comparisons are based on the Student's t-test or analysis of variance. A value of $p < 0.05$ is used to mark a statistically significant difference. All statistical analyses were performed using SigmaStat software (Jandel Scientific, San Rafael, CA).

RESULTS

Character of CEL and its effects on anti-leukemia U937 cells

In Figure 1A, CEL shows hemagglutination activity with a cross-reaction to erythrocytes (type O) until the dilution of $1/2^4$, while the heated CEL (HCEL) markedly decreased in agglutination activity. CEL, but not HCEL, also decreased the viability of U937 cells at concentrations higher than 50 μg/ml (Figure 1B).

The CM collected from CEL- or HCEL-treated MNCs showed that both CEL- and HCEL-MNC-CM had a significant inhibitory effect on human leukemia U937 cells (Figure 1C). PHA (10 μg/ml) was used in this study as the positive control; PHA-treated MNC-CM showed 50.2 ± 4.6% inhibitory activity on U937 cells. In addition, CEL-MNC-CM had a greater effect inducing differentiation of U937 cells to monocytes than HCEL-MNC-CM. This is true not only morphologically (Figure 1D), but also by NBT reduction test (Figure 1E). Therefore, CEL at concentrations lower than 30 μg/ml was used for further experiments. Polymyxin B was added to MNC-CM and did not elicit an effect regarding CEL and HCEL, suggesting non-existent or insignificant endotoxin contamination.

Effect of CEL on MNCs

Figure 2A shows that CEL markedly induced MNCs secretion of cytokines, such as TNF-α and IL-1β. When the treatment of CEL at 30 μg/ml, TNF-α and IL-1β production were markedly increased for 5.3- and 2.0-fold, respectively.

The stimulating effect of TNF-α and IL-1β production in positive control PHA (10 μg/ml) was 984 and 1356 pg/ml, respectively. Monocytes contained in CEL-treated MNCs significantly increased the expression of macrophage morphology, formazan-positive cells, and

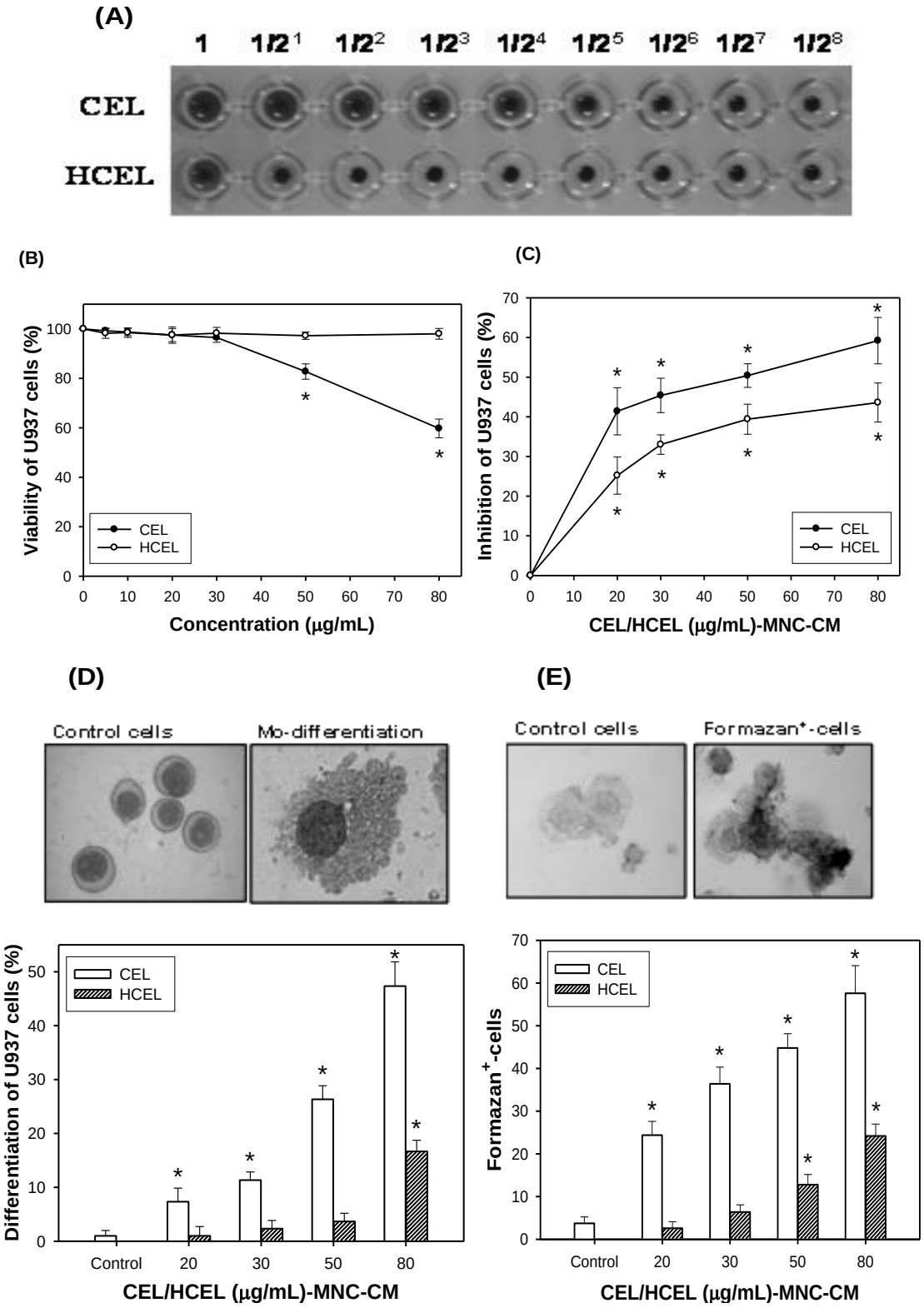

Figure 1. Characteristics of CEL and its effects on anti-leukemia U937 cells. (A) Hemagluttination activity of CEL (initiation concentration of 1 μg/ml) and heat-treated CEL (HCEL) on type O erythrocytes. (B) Viability of U937 cells treated with various concentrations of CEL and HCEL for 24 h and assayed by using the trypan blue dye exclusion test. (C) Growth inhibition, (D) Monocyte (Mo) differentiation, and (E) NBT reduction test of U937 cells with treatment of 30% CEL- and HCEL-mononuclear cell (MNC)-conditioned medium (CM) for 5 days. All data are shown as the mean ± SD for at least three independent experiments. *A value of $p < 0.05$ was chosen to indicate a significant difference compared with the control group.

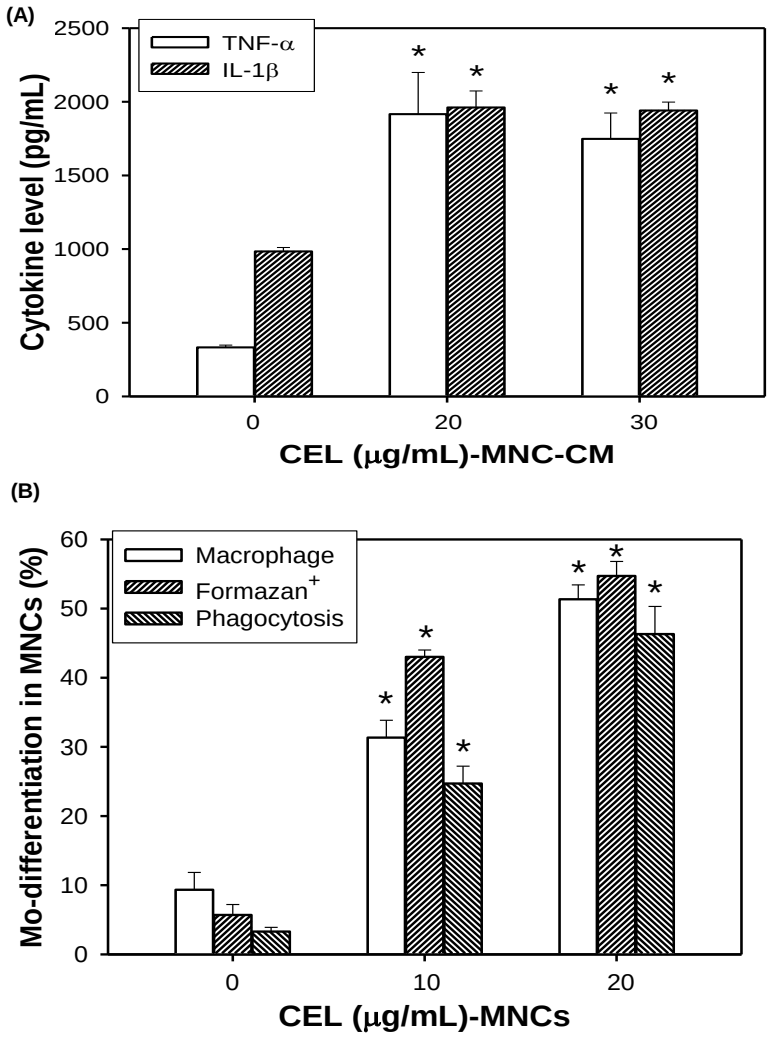

Figure 2. Effects of CEL on MNCs cultured for 24 h. (A) Cytokine production contained in CEL-treated MNCs by ELISA analysis. (B) Differentiation of monocytes (Mo) in MNCs with CEL (0, 10, and 20 μg/ml) treatment. All data are shown as the mean ± SD for at least three independent experiments. *A value of $p < 0.05$ was chosen to indicate a significant difference compared with the control group.

phagocytic activity (Figure 2B).

Effects of CEL on monocytes and lymphocytes

To further examine the effects of CEL on monocytes and lymphocytes, various concentrations of CEL (0 to 30 μg/ml) were treated with monocytes or lymphocytes. The CMs from CEL-treated monocytes (CEL-Mo-CM) were collected and tested their effects on lymphocytes, indicating a marked increase in the viability of lymphocytes (Figure 3A). However, CEL-treated lymphocyte-CM (CEL-Lym-CM) showed that there was no change to the cell viability of monocytes (Figure 3B) and macrophage differentiation (Figure 3C). This suggests that CEL might directly stimulate monocytes, but not lymphocytes, to produce certain cytokines in CEL-Mo-CM. Then, CEL-Mo-CM may have an autocrine and/or paracrine effect to further enhance the viability lymphocytes.

Interaction between monocytes and lymphocytes

Since CEL-Mo-CM effectively activated lymphocytes, there was a significant increase of IFN-γ secretion in CEL-Mo-CM-treated lymphocyte-CM (Mo/Lym-CM) in Figure 4A. Moreover, CEL-Mo-CM-activated lymphocytes

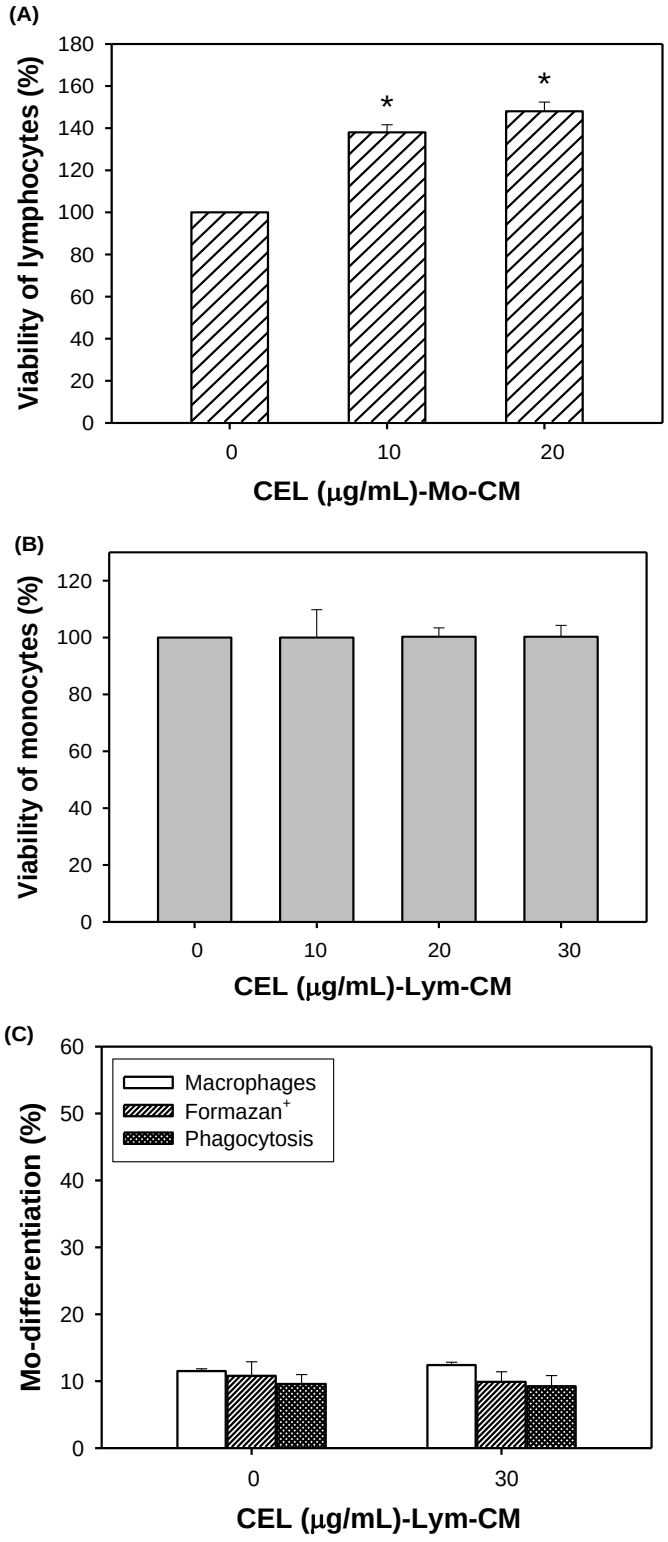

Figure 3. Effects of CEL (0 - 30 μg/ml) on monocytes (Mo) and lymphocytes (Lym) cultured for 24 h. (A) Viability of lymphocytes in MNCs with treatment of 30% CEL-Mo-CM. (B) Viability of monocytes with treatment of 30% CEL-Lym-CM. (C) Effect of 30% CEL-Lym-CM on monocyte differentiation. All data are shown as the mean ± SD for at least three independent experiments. *A value of $p < 0.05$ was chosen to indicate a significant difference compared with the control group.

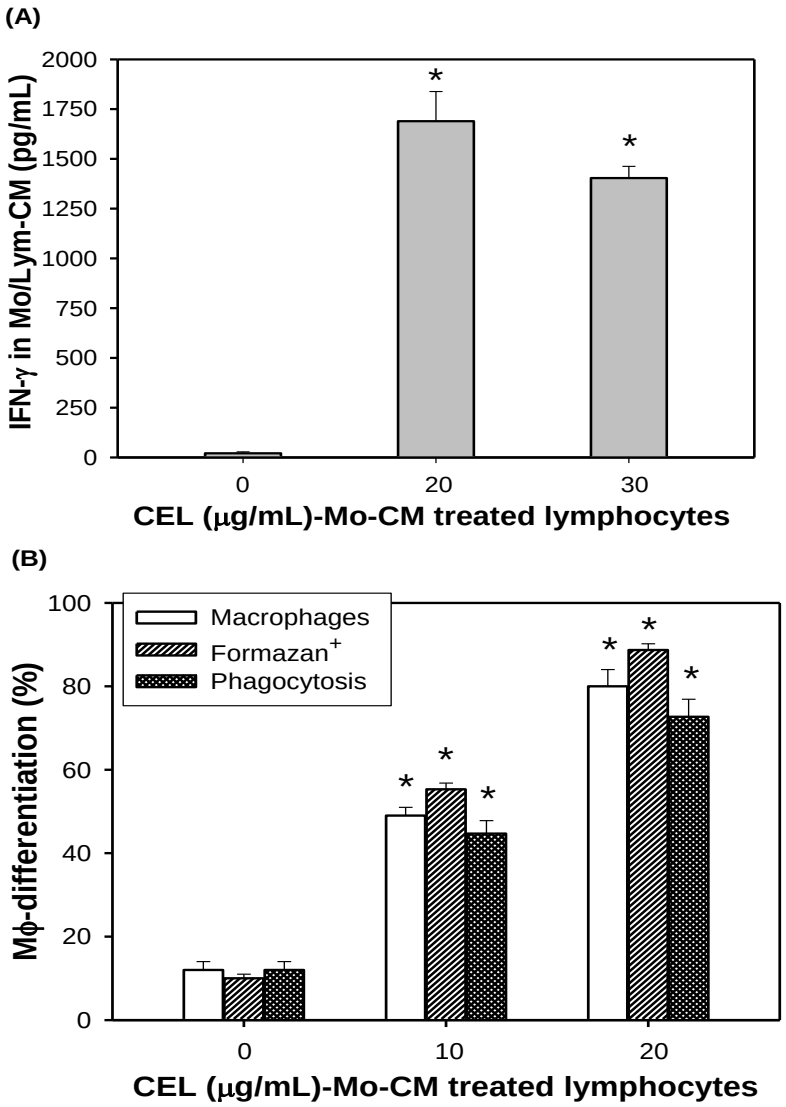

Figure 4. Interaction between monocytes (Mo) and lymphocytes (Lym) undergoing treatment of CEL-Mo-CM for 24 h culturing. (A) Production of IFN-γ in lymphocytes with treatment of 30% CEL-Mo-CM. (B) Macrophage differentiation that activated with CEL-Mo-CM-treated lymphocytes. All data are shown as the mean ± SD for at least three independent experiments. *A value of $p < 0.05$ was considered to indicate a significant difference compared with the control group.

were co-cultured with monocytes, and the result showed a significant increase of macrophage differentiation (Figure 4B). The expression of macrophage morphology, formazan-positive cells, and phagocytic activity was markedly up-regulated (Figure 4B).

Expression of CD40

In Figure 5A, immunofluorescent staining of monocytes with anti-CD40 antibody (red) and Hoechst 33342 (nuclei, blue) indicated that CD40 was clearly expressed with CEL (10 μg/ml) treatment. Figure 5B showed that the CD40 blocking peptide significantly reduced CEL-induced macrophage (Mϕ) activation in formazan-positive level. Additionally, CD40 blocking peptide added in CEL-treated MNC also eliminated the inhibitory rate of CEL-MNC-CM in U937 cells (Figure 5C). This suggests that CEL stimulated monocytes thereby secreting cytokines, such that they not only activated lymphocytes, but also increased CD40 expression in monocytes. The interaction of activated lymphocytes and monocytes would induce macrophage differentiation which may cause anti-leukemia immunity against U937 cells.

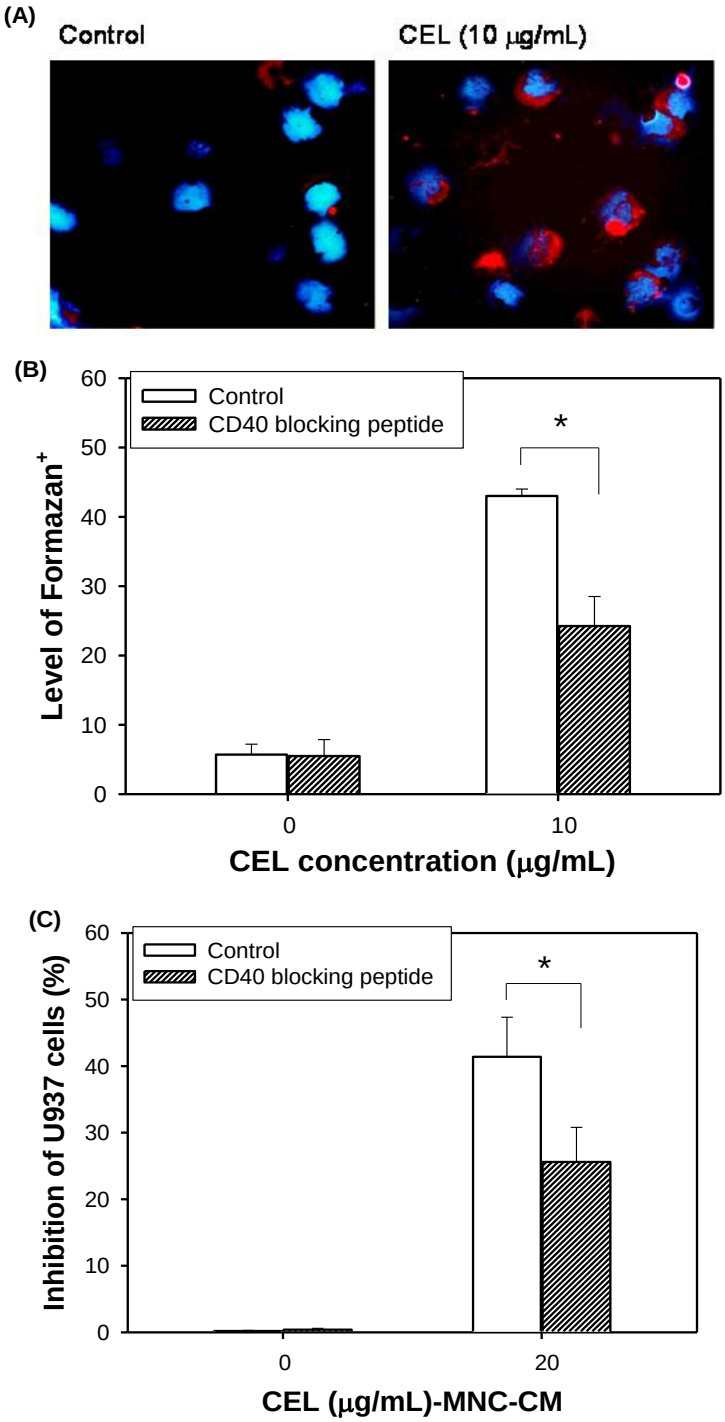

Figure 5. Expression of CD40 in cells with CEL treatment for 24 h. (A) Immunofluorescent staining of monocytes with anti-CD40 antibody (red) and Hoechst 33342 (nuclei, blue). (B) Effects of CD40 blocking peptide on macrophage (Mϕ) activation. (C) Effects of CD40 blocking peptide on CEL-MNC-CM-mediated inhibition of U937 cells. MNCs were treated with CEL (10 μg/ml) in the presence or absence of CD40 blocking peptide (80 ng/ml) and co-cultured for 24 h. Then, the monocytes were separated and CEL-MNC-CM was collected for analysis of NBT reduction test and inhibitory effect of U937 cells, respectively. All data are shown as the mean ± SD for at least three independent experiments. *A value of $p < 0.05$ was considered to indicate a significant difference.

DISCUSSION

Lectins from *C. ensiformis* (CEL) mainly contain Con A which has a molecular weight of 102.5 kDa and consists of four subunits (tetramer) (Agrawal and Goldstein, 1968). Con A, which possesses agglutinin specificity, has been reported to stimulate innate immunity and TLR expression in macrophages (Sodhi et al., 2007; Conchon-Costa et al., 2007) and induce proliferation of peripheral blood mononuclear cells (Renner et al., 2011). This study has demonstrated that CEL exhibited agglutination activity to type O erythrocytes, while heat treatment of CEL at 100°C for 5 min markedly decreased this effect. The result was suggested that CEL may not stable in high temperature condition. CEL concentrations higher than 30 μg/ml directly inhibited the growth of human leukemia U937 cells. However, lower concentrations (less than 30 μg/ml) of CEL effectively stimulated mononuclear cells (MNCs) from human peripheral blood by secreting cytokines, such as TNF-α and IL-1β in their conditioned medium (CM) which caused inhibited growth and differentiation induction in U937 cells. Our previous studies have developed the model of anti-leukemic immunity, and assessed the natural products, such as polysaccharide from black bean (Liao et al., 2001; Liao et al., 2005) and rice prolamin (Liao et al., 2006), on stimulating MNCs to produce various cytokines for inducing the growth inhibition and differentiation of tumor cells. Therefore, we used CEL at a concentration lower than 30 μg/ml and investigated its regulatory effect between immunocompetent cells, monocytes and lymphocytes.

The cytokines produced in CEL-treated MNC-CM showed a significant increase in TNF-α and IL-1β, which were secreted from macrophages to induce an inflammatory effect and activate other immune cells, such as lymphocytes (de Pádua Queiroz et al., 2010). Further investigation of the cells contained in CEL-treated MNCs demonstrated that macrophage morphology and function including superoxide production and phagocytosis were significantly increased. Unlike PHA, a positive control used in this study, lectins exhibit potent mitogenic activity towards T lymphocytes (Kinikli et al., 2005). This result suggests that CEL may target monocytes and induce differentiation toward terminal macrophages.

We separated monocytes and lymphocytes from MNCs and then these two type cells were treated with CEL, demonstrating that CEL-Mo-CM significantly stimulated the viability of monocytes. CEL-Lym-CM did not change monocyte viability and macrophage differentiation. Interestingly, lymphocytes treated with CEL-Mo-CM significantly increased the level of IFN-γ, a type of cytokine secreted by T lymphocytes and natural killer cells for activating macrophages. In the previous study, for example, rice bran (MGN-3/Biobran) has been reported that augments IFN-γ secretion from human peripheral blood lymphocytes (Ghoneum and Jewett, 2000) and can enhance the phagocytic activity of macrophages (Ghoneum and Matsuura, 2004). Therefore, the data revealed that CEL may first activate monocytes to secrete TNF-α and IL-1β, and then stimulate lymphocytes to produce IFN-γ. However, the effect of macrophage terminal differentiation is induced by CEL-Mo-CM-treated lymphocytes, as opposed to their conditioned medium (Mo-Lym-CM) alone, strongly indicating a cell-to-cell interaction between the activated lymphocytes and macrophages.

Human peripheral $CD14^+$ monocytes have been known to differentiate into monocyte-derived macrophages or dendritic cells upon suitable stimulation for PKC-alpha and PKC-betaI translocation (Lin et al., 2007). Wang et al. (2010) reported that andrographolide modulates the innate and adaptive immunities by regulating macrophage phenotype and costimulatory molecular (such as CD40) expression, inducing an antigen-specific immune response in lymphocytes. In this study, an advanced assessment of CD40 expression in monocytes from CEL-treated MNCs presented results that were markedly up-regulated. Treatment with the CD40 blocking peptide significantly reduced the activation of macrophages by NBT reduction test. As such, this study has demonstrated that the macrophage differentiation effect of CEL first stimulates monocytes to produce cytokines (such as TNF-α and IL-1β) to activates lymphocytes, and then stimulates CD40 expression on the surface of monocytes. Finally, the macrophage terminal differentiation may take place through a cell-to-cell interaction between the activated lymphocytes and monocytes.

Additionally, CD40 blocking peptide added in CEL-treated MNC also eliminated the inhibitory rate of CEL-MNC-CM in leukemic U937 cells. Previous articles have reported that polysaccharides or saccharide-linked lectins from natural products were effective in antitumor immunity through the activation of immunocompetent cells against tumor growth (Chen and Chang, 2004; Liao et al., 2001) as well as promoting myelopoiesis in bone marrow suppressive mice (Liao et al., 2005). In this study, we conclude that CEL induces macrophage differentiation through the indirect modulation of lymphocytic activity, and importantly, induced an anti-leukemia immunity effect.

ACKNOWLEDGEMENTS

This study was supported by grant NSC 100-2313-B-415-001 from the National Science Council of Republic of China, Taipei, Taiwan. This paper has been edited by a native English speaker prior to submission.

REFERENCES

Agrawal BB, Goldstein IJ (1968). Protein–carbohydrate interactin.

XV. The role of bivalent cations in concanavalin A-polysaccharide interaction. Can. J. Biochem., 46: 1147-1150.
Berezhnaya NM, Belova OB, Vinnichuk YD, Tarutinov VI (2009). Expression of E-cadherin in drug resistant human breast cancer cells and their sensitivity to lymphokine-activated lymphocytes action. Exp. Oncol., 31: 42-245.
Beuth I, Ko HL, Gabius HJ, Pulverer G (1991). Influence of treatment with the immunomodulatory effective dose of the P-galactoside-specifit lectin from mistletoe on tumor colonization in BALB/c- mice for two experimental model systems. *In vivo* 5: 29-32.
Beuth J, Ko HL, Gabius HJ, Butrichter H, Oette K, Pulverer G (1992). Behaviour of lymphocyte subsets and expression of activation markers in response to immunotherapy with galactoside-specific lectin from mistletoe in breast cancer patients. Clin. Inves., 70: 658-661.
Boccil V (1993). Mistletoe (*Viscum album*) lectins as cytokine inducers and immunoadjuvant in tumor therapy. J. Biol. Regul. Homeost. Agent. 7: 1-6.
Chen YY, Chang HM (2004). Antiproliferative and differentiating effects of polysaccharide fraction from Fu-Ling (*Poria cocos*) on human leukemic U937 and HL-60 cells. Food Chem. Toxicol., 42: 759-769.
Conchon-Costa I, Loyola W, Gaziri LC, Custodio LA, Felipe I (2007). Low dose of Concanavalin-A enhances innate immune response and prevents liver injury in mice infected with Candida albicans. FEMS Immunol. Med. Microbiol., 49: 330-336.
de Pádua QL Jr, Mattos ME Jr, da Silva MF, Silva CL (2010). TGF-beta and CD23 are involved in nitric oxide production by pulmonary macrophages activated by beta-glucan from Paracoccidioides brasiliensis. Med. Microbiol. Immunol., 199: 61-69.
Gabius HJ, Gabius S, Joshi SS, Koch B, Schroeder M, Manzke W, Westerhausen M (1994). From ill-defined extracts to the immunomodulatory lectin: Will there be a reason for oncological application of mistletoe? Planta Med., 60: 2-7.
Ghoneum M, Jewett A (2000). Production of tumor necrosis factoralpha and interferon-gamma from human peripheral blood lymphocytes by MGN-3, a modified arabinoxylan from rice bran, and its synergy with interleukin-2 *in vitro*. Cancer Detect. Prevent., 24: 314-324.
Ghoneum M, Matsuura M (2004). Augmentation of macrophage phagocytosis by modified arabinoxylan rice bran (MGN-3/biobran). Int. J. Immunopathol. Pharmacol., 17: 283-292.
Hirano K, Teraoka T, Yamanaka H, Harashima A, Kunisaki A, Takahashi H, Hosokawa D (2000). Novel mannose-binding rice lectin composed of some isolectins and its relation to a stress-inducible salT gene. Plant Cell Physiol., 41: 258-267.
Jeon KS, Na HJ, Kim YM, Kwon HJ (2005). Antiangiogenic activity of 4-O-methylgallic acid from *Canavalia gladiata*, a dietary legume. Biochem. Biophys. Res. Commun., 330: 1268-1274.
Joshi SS. Tomes DJ, Pirruccello SJ, Gabius HJ (1994). Galactoside specific VAA lectin-induced apoptosis in human leukemia cells. Proc. Am. Assn. Cancer Res., 35: 6.
Kesherwani V, Sodhi A (2007). Differential activation of macrophages in vitro by lectin Concanavalin A, Phytohemagglutinin and Wheat germ agglutinin: production and regulation of nitric oxide. Nitric Oxide, 16: 294-305.
Kinikli S, Turkcapar N, Kucukay MB, Keskin G, Kinikli G (2005). In vitro nonspecific mitogenic response of T-cell subsets in acute and chronic brucellosis. Diagn. Microbiol. Infect. Dis., 52: 229-233.
Liao HF, Chen YJ, Yang YC (2005). A novel polysaccharide of black soybean promotes myelopoiesis and reconstitutes bone marrow after 5-flurouracil- and irradiation-induced myelosuppression. Life Sci., 77: 400-413.
Liao HF, Chen YY, Yang YC, Wang CS, Chen YJ (2006). Rice (*Oryza sativa* L.) inhibits growth and induces differentiation of human leukemic U937 cells through activation of peripheral blood mononuclear cells. Food Chem. Toxicol., 44: 1724-1729.
Liao HF, Chou CJ, Wu SH, Khoo KH, Chen CF, Wang SY (2001). Isolation and characterization of an active compound from black soybean [*Glycine max* (L.) Merr.] and its effect on proliferation and differentiation of human leukemic U937 cells. Anticancer Drug. 12: 841-846.
Lin YF, Lee HM, Leu SJ, Tsai YH (2007).The essentiality of PKCalpha and PKCbetaI translocation for $CD14^{+}$monocyte differentiation towards macrophages and dendritic cells, respectively. J. Cell. Biochem., 102: 429-441.
Martínez-Cruz M, Zenteno E, Córdoba F (2001). Purification and characterization of a galactose-specific lectin from corn (*Zea mays*) coleoptile. Biochim. Biophys. Acta., 1568: 37-44.
Masnikosa R, Baricevic I, Lagundzin D, Nedic O (2010). Characterisation of N-glycans bound to IGFBP-3 in sera from healthy adults. Biochimie., 92: 97-101.
Mody R, Joshi S, Chaney W (1995). Use of lectins as diagnostic and therapeutic tools for cancer. J. Pharmacol. Toxicol. Method, 33: 1-10.
Monsigny M, Roche AC, Sene C, Maget-Dana R, Delmotte F (1980). Sugar-lectin interactions: how does wheat-germ agglutinin bind sialoglycoconjugates? Eur. J. Biochem., 104: 147-153.
Murakami T, Kohno K, Kishi A, Matsuda H, Yoshikawa M (2000). Medicinal Foodstuffs. XIX. Absolute Stereostructures of Canavalioside, a New *Ent*-Kaurane-Type Diterpene Glycoside, and Gladiatosides A1, A2, A3, B1, B2, B3, C1, and C2, New Acylated Flavonol Glycosides, from Sword Bean, the Seeds of *Canavalia gladiata*. Chem. Pharm. Bull., 48: 1673-1680.
Nehmann N, Schade UM, Pfüller U, Schachner M, Schumacher U (2009). Mistletoe lectin binds to multidrug resistance-associated protein MRP5. Anticancer Res., 29: 4941-4948.
Peterslund NA, Koch C, Jensenius JC, Thiel S (2001). Association between deficiency of mannose-binding lectin and severe infections after chemotherapy. Lancet, 358: 637-638.
Prodanov P, Atanasova N (1984). An anti-B lectin from *Zea mays* everta. Folia Haematol., 111: 84-85.
Renner L, Schwabe A, Döll S, Höltershinken M, Dänicke S (2011). Effect of rare earth elements on beef cattle growth performance, blood clinical chemical parameters and mitogen stimulated proliferation of bovine peripheral blood mononuclear cells in vitro and ex vivo. Toxicol. Lett., 201: 277-284.
Sharon N, Lis H (1989). Lectins as cell recognition molecules. Science, 246: 227-234.
Sodhi A, Tarang S, Kesherwani V (2007). Concanavalin A induced expression of Toll-like receptors in murine peritoneal macrophages *in vitro*. Int. Immunopharmacol., 7: 454-463.
Suzuki O, Nozawa Y, Abe M (2007). Loss of L-PHA-, PNA-, or ConA-reactive oligosaccharides is associated with a poor prognosis in human Burkitt's lymphoma. Oncol. Rep., 17: 775-779.
Wang W, Wang J, Dong SF, Liu CH, Italiani P, Sun SH, Xu J, Boraschi D, Ma SP, Qu D (2010). Immunomodulatory activity of andrographolide on macrophage activation and specific antibody response. Acta. Pharmacol. Sin., 31: 191-201.
Yu L, Femig DG, Smith JA, Milton JD, Rhodes JM (1993). Reversible inhibition of proliferation of epithelial cell lines by *Agaricus bisporus* (edible mushroom) lectin. Cancer Res., 53: 4627-4632.