Food Sci. Biotechnol. Vol. 17, No. 3, pp. 631 ~ 636 (2008)

Food Science and Biotechnology

The Korean Society of Food Science and Technology

Th1/Th2 Cytokine Modulation in Human PBMC by *Acanthopanax divaricatus* var. *albeofructus*

Su-Yun Lyu[1] and Won-Bong Park*

College of Natural Sciences, Seoul Women's University, Seoul 139-774, Korea

[1]Department of Herbal Medicinal Pharmacology, Daegu Haany University, Gyeongsan, Gyeongbuk 712-715, Korea

Abstract *Acanthopanax divaricatus* var. *albeofructus* (ADA) have been shown to have various levels of activity such as antioxidant, anticancer, antivirus, and immunostimulatory effects. However, little is known about its mechanism related to the modulation of immune activities. In this study, a water extract of ADA leaves were used to treat human peripheral blood mononuclear cells (hPBMC) to determine the underlying mechanisms for the immunostimulatory effects. To characterize its immunomodulatory activity, the secretion level of various cytokines including IL-2, IL-4, IL-6, IL-10, IL-12, IFN-γ, and TNF-α were measured using enzyme-linked immunosorbent assay (ELISA). Treatment of hPBMC with ADA leaf extract in an *in vitro* experiment induced various Th1 cytokines in a dose-dependent manner. A significant increase of IL-2, IL-12, IFN-γ, and TNF-α secretion was observed in the presence of ADA leaf extract. In contrast, Th2 cytokines including IL-4 and IL-6 were suppressed. There was no significant change in IL-10 release. Our results showed an increase in Th1 and a decrease in Th2 cytokine secretion which suggests that ADA may influence the immune response towards a predominance of Th1 cytokines in the immune system.

Keywords: *Acanthopanax*, cytokine, hPBMC, immunity

Introduction

Acanthopanax species (Araliaceae) are widely distributed in Korea, China, Japan, and the far-eastern region of Russia (1-3). The whole plant including leaves, stem, and roots has been widely taken as health supplements in Korea, for instance as antirheumatoid arthritis, antiinflammatory, and antidiabetic drugs and are also recognized to have ginseng-like activities (4,5). There are 18 kinds of plants belonging to the genus *Acanthopanax* in Korea including 10 species, 5 forma, and 3 varieties (6). Traditional medicine is becoming an increasingly attractive approach for the treatment of various inflammatory disorders among patients unresponsive to or unwilling to take standard medication (7). Several components including phenylpropanoid, lignan, terpenoid, chiisanoside, and acanthoic acid have been previously isolated from *Acanthopanax divaricatus* var. *albeofructus* (ADA) (8), which have been shown to have various levels of activity such as antioxidant, anticancer, antivirus, and immunostimulatory effects (4,9-11). However, little is known about its mechanism related to the modulation of immune activities.

Cytokines are small proteins (approximately 25 kDa) that are released by various cells in the body, usually in response to an activating stimulus, and they induce responses through binding to specific receptors. The cytokines are a structurally diverse group of molecules that include interleukins (ILs), interferons (IFNs), and chemokines (12). They are crucial to innate and adaptive inflammatory responses, cell growth and differentiation, cell death, angiogenesis, and developmental as well as repair processes (13,14). The selective production of type 1 T helper (Th1) cells leads to cell-mediated immunity with normal or increased level of IL-2, IFN-γ, tumour necrosis factor (TNF)-α, and/or IL-12 while the production of predominantly type 2 T helper (Th2) cells provides humoral immunity with an increase in one or more B-cell activities (e.g., hypergammaglobulinemia, autoantibody production, or hyper-IgE) and an increase in the level of IL-4, IL-5, IL-6, IL-10, and/or IL-13 (15). Recently, it has been demonstrated that the immune balance (Th1/Th2 balance) controlled by cytokines produced by Th1 and Th2 plays an important role in immunoregulation, including antitumour immunity (16). Judging by the fact that IL-12, a Th1-activating cytokine, shows a strong antitumour activity *in vivo*, Th1-dominant immunity is superior to Th2 immunity in the induction of antitumour immunity (17).

In this study, a water extract of ADA leaves were used to treat human peripheral blood mononuclear cells (hPBMC) to determine the underlying mechanisms for the immunostimulatory effects. To characterize its immunomodulatory activity, the secretion level of various cytokines including IL-2, IL-4, IL-6, IL-10, IL-12, IFN-γ, and TNF-α were measured using enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.

Materials and Methods

Preparation of *Acanthopanax divaricatus* var. *albeofructus* leaf extract Dried leaves of ADA were purchased from Susin Ogapy Co. (Chunahn, Chungnam, Korea). Two-hundred g of ADA was steeped and stirred in 1 L of distilled water at 4°C overnight, then homogenized for 15 min. The extract was filtered with Whatman filter paper (No. 1, Whatman, Kent, UK) and concentrated with a

*Corresponding author: Tel: +82-2-970-5655; Fax: +82-2-975-3159
E-mail: wbpark@swu.ac.kr
Received October 8, 2007; Revised November 26, 2007;
Accepted November 26, 2007

spray dryer (Niro Inc., Columbia, MD, USA). The resulting powder (25 g) was diluted with distilled water and filtered step by step through various membrane filter sizes (0.8, 0.45, and 0.2 μm; Nippon Millipore Ltd., Tokyo, Japan). The sample was stored in −80°C until use.

Cell culture To prepare peripheral blood mononuclear cells (PBMC), human peripheral blood (30 mL) was obtained from healthy human volunteers (age ranging from 25 to 40 years) with the addition of 100 unit/mL heparin. PBMC was isolated by the Histopaque gradient density method according to the manufacturer's instructions (Sigma-Aldrich, Poole, UK). The numbers of PBMC were counted and the cell suspension was adjusted to 1×10^6 cells/mL. Cell numbers and viability were assessed by trypan blue (Sigma-Aldrich) dye exclusion. All cultures were carried out in complete tissue culture medium (CTCM), RPMI-1640 medium (Sigma-Aldrich) supplemented with 5% fetal calf serum (FCS, Sigma-Aldrich), 100 U/mL penicillin/100 μg/mL streptomycin (Sigma-Aldrich) 2 mM L-glutamine (Sigma-Aldrich), and 5×10^{-5} M 2-mercaptoethanol (2-ME, Sigma-Aldrich). Cell cultures were incubated at 37°C in a humidified, 5% CO_2 atmosphere.

Cytotoxicity assay Cytotoxicity assays measure drug-induced alterations in metabolic pathways or structural integrity which may or may not be related directly to cell death. *In vitro* cell viability was measured using solutions of a novel tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethyoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS, Promega, Southampton, UK] and an electron coupling reagent (phenazine methosulphate; PMS, Promega) which facilitates the reduction of MTS. Briefly, cells (5×10^5 cells/mL) were added in 96-well flat-bottomed tissue culture plates (NUNC, Life Technologies, Paisley, UK) in the absence or presence of ADA solution at various concentrations for 48 hr at 37°C in a humidified, 5% CO_2 atmosphere. At the end of the incubation, 40 μL of combined MTS/PMS solution was added into each well of the 96 well assay plate containing 100 μL of cells and 100 μL of samples in culture medium. The plate was incubated for 4 hr at 37°C in a humidified, 5% CO_2 atmosphere. The production of formazan was determined by measuring the absorbance of the compound at 450 nm with a spectrophotometric 96-well plate reader (Dynex Technologies, Chantilly, VA, USA). The viability of the cells was calculated as: % viability = (absorbance of treated cells/absorbance of untreated cells)×100.

Cytokine secretion For cytokine production, cells (1×10^5 cells/well) were seeded in 96-well NUNC (Life Technologies) in the absence or presence of various ADA concentrations for 48 hr. ELISA was applied for qualitative and quantitative determinations of cytokines in the culture supernatants. For the determination of cytokines, 96-well NUNC MaxiSorp (Life Technologies) plates were coated with purified anti-cytokine capture antibody (BD Biosciences, San Jose, CA, USA) in binding buffer (0.1 M Na_2HPO_4, adjusted to pH 9.0 with 0.1 M NaH_2PO_4) overnight at 4°C. After washing the plates 3 times with phosphate buffered saline (PBS)-Tween, which contained PBS with 0.5%(v/v) Tween 20 (Sigma-Aldrich), the plates were blocked with 1%(w/v) bovine serum albumin (BSA) (Sigma-Aldrich) at room temperature for 2 hr. Following 3 washes with PBS-Tween, cell culture supernatants were added and incubated overnight at 4°C; recombinant cytokines (BD Biosciences) were included as standards for each plate. After 4 washes with PBS-Tween, the 'captured' cytokine was sandwiched with biotinylated antibody (BD Biosciences) and incubated at room temperature for 1 hr. Following 4 washes, the presence of biotinylated antibodies was detected with streptavidin-peroxidase (BD Biosciences). At the end of 1 hr incubation at room temperature, the assay was developed using 0.1 mg/mL of 3,3',5,5'-tetramethylbenzidine substrate (Sigma-Aldrich) in 0.05 M phosphate-citrate buffer (pH 5.0), containing 0.03% hydrogen peroxide. The enzyme reaction was stopped with 2.5 M sulphuric acid after an incubation of 10 min at room temperature, the colourimetric development was read at 450 nm with a spectrophotometric 96-well plate reader (Dynex Technologies). The concentrations of cytokines in the culture supernatants were determined by extrapolation from the reference standard curve.

Statistical analysis Student paired two-tailed *t*-test was used for comparisons (MINITAB®, release 14.1, Minitab Inc.). Probability values (*p*-value) of <0.001, <0.01, or <0.05 were considered significant with 99.9, 99, or 95% of confidence, respectively.

Results and Discussion

Cytotoxicity of ADA leaf extract In order to discern whether inhibition of cytokine secretion was due to cytostatic or cytotoxic effect, and in order to verify that the administration dose had minimal effect on the cells, the viability of PBMC was measured 48 hr post-treatment. The cells were treated for 48 hr with ADA leaf extract at the indicated dose and the viability was measured using the MTS assay. Human PBMC showed more than 95% of the cells survived at concentrations lower than 10^{-5} g/mL, which is the highest concentration used in cytokine assay (Fig. 1). Therefore we chose concentrations lower than 10^{-5} g/mL for further experiments to ensure that ADA leaf extract has no major effect on the cells when compared to the controls.

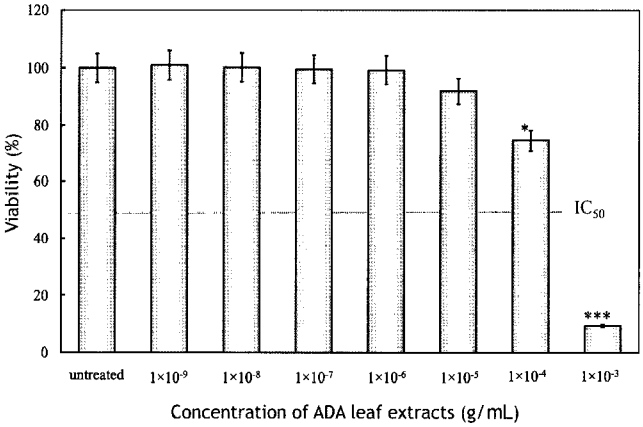

Fig. 1. Determination of ADA leaf extract cytotoxicity against hPBMC using MTS assay. Cells were added in 96-well flat-bottomed tissue culture plates in the absence or presence of ADA leaf extract at various concentrations. ***$p<0.001$, *$p<0.05$.

Effect of ADA leaf extract on Th1 cytokine secretion Recently, it was reported that water-extracted *Acanthopanax senticosus* and stimulated peritoneal macrophage, which was followed by the production of various cytokines such as IL-1β, IL-12, IFN-γ, and TNF-α (7,18). The next step was to investigate whether ADA as an immunomodulator could lead to increased cytokine production associated with cell-mediated immunity. The parameters selected were Th1 cytokines (IL-2, IL-12, IFN-γ, and TNF-α) which are considered pro-inflammatory cytokines (19). Treatment of hPBMC with ADA leaf extract in an *in vitro* experiment induced various Th1 cytokines in a dose-dependent manner. An increase of IL-2 (ADA concentration range of 10^{-18} -10^{-17} and 10^{-13}-10^{-7} g/mL, Fig. 2A), IL-12 (10^{-16}-10^{-6} g/mL, Fig. 2B), IFN-γ (10^{-11}-10^{-7} g/mL, Fig. 2C), and TNF-α (10^{-13}-10^{-8} g/mL, Fig. 2D) secretion was observed in the presence of ADA leaf extract compared with the untreated cells. The most significant change ($p<0.001$) for IL-2, IL-12, IFN-γ, and TNF-α secretion was when the cells were treated with 10^{-9}-10^{-8}, 10^{-11}, 10^{-9}, and 10^{-11}-10^{-10} g/mL, respectively. The negative control (RPMI 1640+5% FCS; CTCM) did not exert any effect on the release of cytokines.

Effect of ADA leaf extract on Th2 cytokine secretion Further studies were carried out to determine if Th1 cytokine secretion exhibited by ADA leaf extract correlated with Th2 cytokine secretion. The parameters selected were IL-4, IL-6, and IL-10 which are anti-inflammatory cytokines that promote humoral immunity and tolerance (22). As presented in Fig. 3 and 4, treatment of hPBMC with ADA leaf extract suppressed Th2 cytokines including IL-4 (ADA concentration range of 10^{-21}-10^{-5} g/mL, Fig. 3A) and IL-6 (10^{-17}-10^{-12} g/mL, Fig. 3B). The most significant change ($p<0.001$) for IL-4 and IL-6 secretion was when the cells were treated with 10^{-14} and 10^{-14}-10^{-13} g/mL of ADA leaf extract, respectively. These are slightly lower than the optimal concentrations that can induce IL-2, IL-12, IFN-γ, and TNF-α secretion (10^{-11}-10^{-8} g/mL, see above). IL-4 directs the development of Th2 cells both *in vitro* and *in vivo*, and downregulates IL-2 and IFN-γ production in Th1 cells (23). Therefore, the increase of IL-2 and IFN-γ may be aided by the suppression of IL-4 secretion in hPBMC. On the other hand, there was no statistical difference in IL-10 secretion (Fig. 3C) between the treatment and the control group ($p>0.05$). The negative control (RPMI 1640+5% FCS; CTCM) did not exert any effect on the release of cytokines.

Among *Acanthopanax* species, *A. senticosus* has been well-known as an adaptogenic medicine, but this plant is endangered by over-harvesting and exacerbated by its slow growth. Other species such as *A. divaricatus* var. *albeofructus*, *A. chiisanensis*, and *A. koreanum* are well cultivated in southern Korea and it may well be possible to substitute the extract of *A. senticosus* on the basis of superiority in their constituents and biological activities (21). *A. divaricatus* var. *albeofructus* has been used in Korea as a tonic and sedative as well as a drug with ginseng-like activities (4).

Oriental medicines, cytokines, and synthetic adjuvants have shown biological activities that enhance the host defense system and have been applied for cancer immunotherapy (22-24). Especially, traditionally used natural resources for stimulating the immune system have emerged as important molecules for cancer therapy (24, 25). In the present investigation, we demonstrated that ADA modulates the pattern of Th1 (IL-2, IL-12, IFN-γ, and TNF-α) and Th2 cytokine (IL-4, IL-6, and IL-10) secretion in human PBMC which can be divided into 75% of T cells bearing T cell receptors (detected with anti-CD3 antibodies), 15% of B cells bearing immunoglobulin receptors (detected with anti-immunoglobulin antibodies), and null cells including natural killer (NK) cells, that label with neither. Cytokines are major mediators of immune response against microorganisms, tumours, and self-antigens. They are a major focus in the study of the pathogenesis of immune responses. Cytokines are produced by a wide variety of cell types including type 1 and type 2 T helper cells (26). In this study, the secretion of Th1 cytokines, such as IL-2 which antagonize the production of type 2 cytokines, were found to be statistically increased compared with the controls. In contrast, the secretion of certain Th2 cytokines, such as IL-4 and IL-6, were decreased. No statistical difference was observed in the secretion of IL-10 between treated and non-treated cells.

The cytokines elicited by infection agents (principally IFN-γ, IL-12, and IL-4), the co-stimulators used to drive the response, and the nature of the peptide:master histocompatibility complex (MHC) ligand all have an effect on the factors that determine whether a proliferating $CD4^+$ T cell will differentiate into a Th1 or a Th2 cell (27). $CD8^+$ T cells may regulate the development of $CD4^+$ helper T cells by producing IFN-γ or IL-12 which suppress the development of Th2 cells and favour Th1 cell growth (28). These cytokines induce the generation of their own T-helper subset, and simultaneously inhibit the production of the opposing subset. Therefore, it is believed that an increase in IFN-γ or IL-12 shifts the Th1/Th2 balance to predominantly Th1 (29). ADA significantly increased IFN-γ and IL-12 secretion in PBMC compared to the controls. Therefore, it can be suggested that ADA may induce T cell differentiation to Th1 cells through the modulation of Th1/Th2 balance by up-regulating Th1 response. Further study to decide if the ratio of $CD4^+$/$CD8^+$ T cells in human PBMC is related to Th1-dominant immunity is needed.

Recently, it has been accepted that the Th1/Th2 balance regulated by 2 distinct Th2 subsets is critical in the onset of various immune diseases (16,20). Nishimura *et al.* (30,31) showed that mice cured of tumour by Th1 cell therapy acquire an immunological memory allowing cytotoxic T lymphocyte (CTL) generation, but this did not occur in mice treated with Th2 cell therapy. They also demonstrated that IL-12 and IL-2 genes are useful to induce tumour rejection through the activation of Th1-dominant immunity. In addition, it was reported that Th2 cells that produce high levels of IL-6 and IL-10 causes cachexia in tumour-bearing hosts (32) which also supports the fact that Th1-dominant immunity is superior to Th2 immunity for application in tumour immunotherapy. Our results showed an increase in Th1 and a decrease in Th2 cytokine secretion which suggests that ADA may influence the immune response towards a predominance of Th1 cytokines in the immune system.

In addition, the activation of T cells by antigen-presenting cells leads to their proliferation and the differentiation of their progeny into armed effector T cells. This depends on

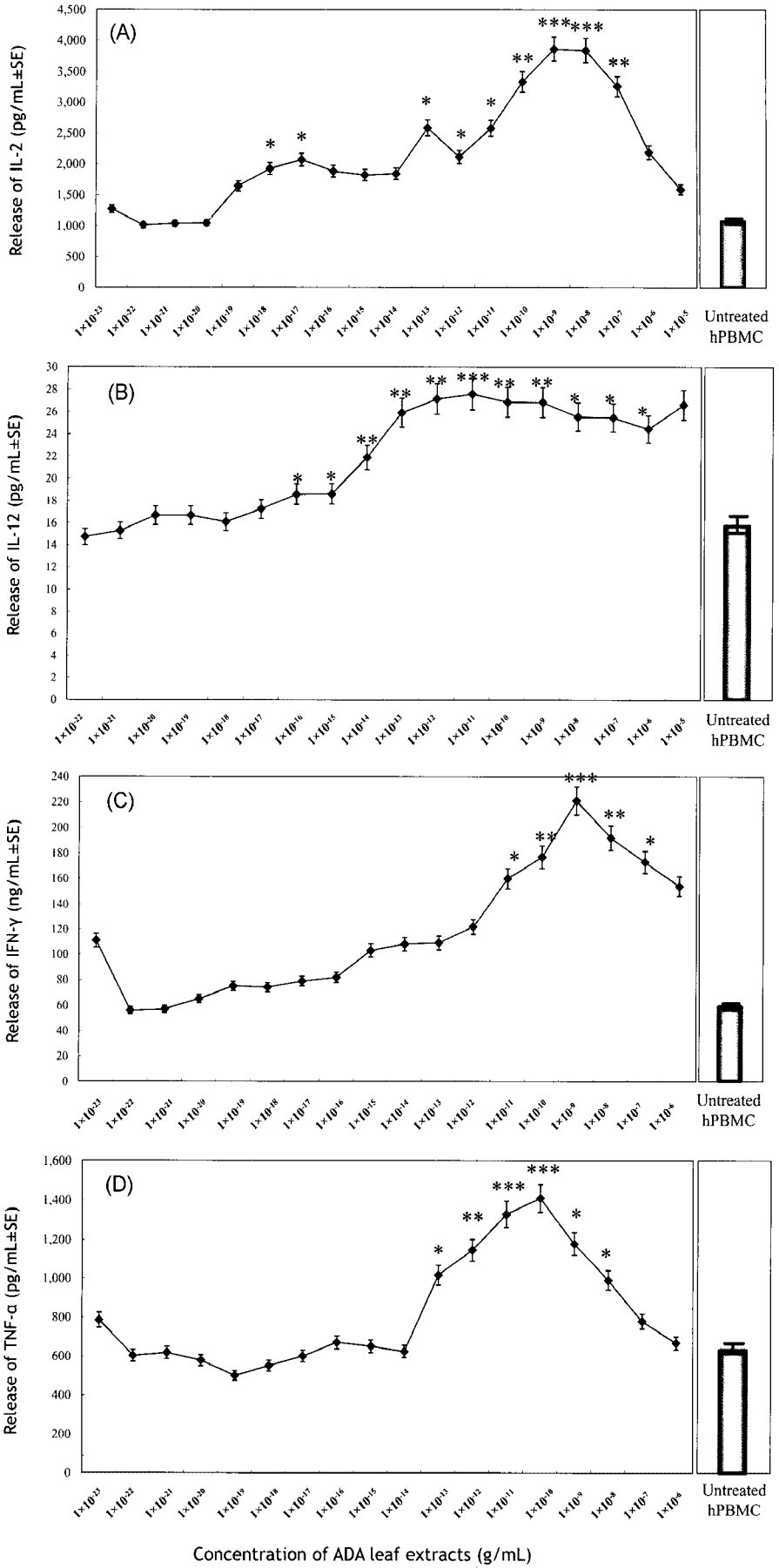

Fig. 2. Effect of ADA leaf extract on Th1 cytokine secretion in hPBMC. Human PBMC in RPMI 1640 (5%) was treated with the indicated concentrations of ADA leaf extract. After 48 hr of incubation at 37°C, released cytokines; IL-2 (A), IL-12 (B), IFN-γ (C), and TNF-α (D), were measured in the cell-free supernatants by ELISA. ***$p<0.001$, **$p<0.01$, *$p<0.05$.

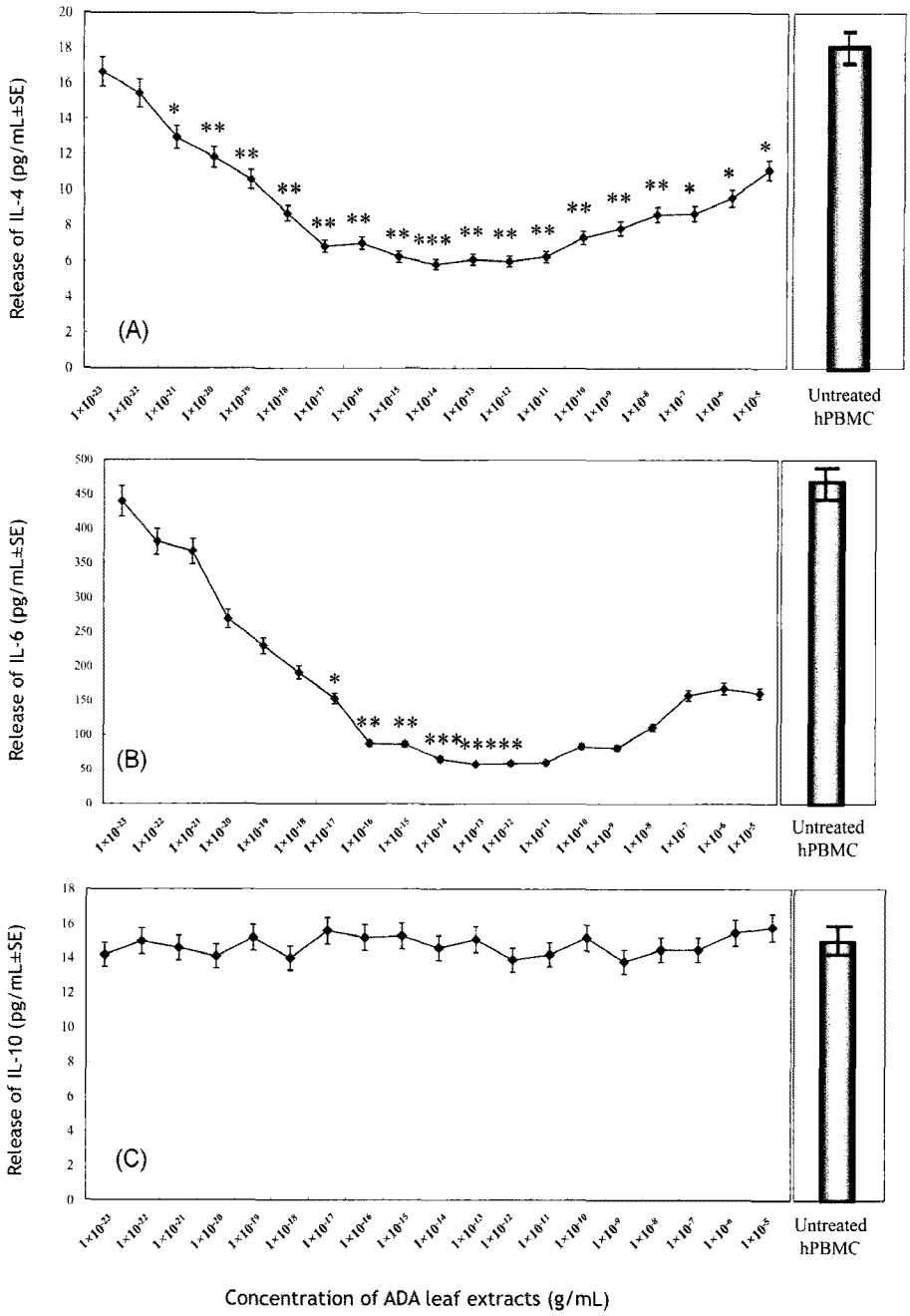

Fig. 3. Effect of ADA leaf extract on Th1 cytokine secretion in hPBMC. Human PBMC in RPMI 1640 (5%) was treated with the indicated concentrations of ADA leaf extract. After 48 hr of incubation at 37°C, released cytokines; IL-4 (A), IL-6 (B), and IL-10 (C), were measured in the cell-free supernatants by ELISA. ***$p<0.001$, **$p<0.01$, *$p<0.05$.

the production of cytokines, in particular the T cell growth factor IL-2, which binds to a high-affinity receptor on the activated T cell. Proliferating T cells develop into armed effector T cells, the critical event in most adaptive immune responses. Effector T cells can mediate a variety of functions. Their most important functions are the killing of infected cells by CD8 cytotoxic T cells and the activation of macrophages by Th1 cells, which together make up cell-mediated immunity (33). The investigation of ADA-induced pro-inflammatory cytokines is important because cancer patients often show decreased inflammatory responsiveness.

Acknowledgment

This work was supported by a special research grant from Seoul Women's University in the program year of 2008.

References

1. Perry LM, Metzger J. Medicinal Plants of East and Southeast of Asia. MIT Press, Cambridge, MA/London. pp. 41-42 (1981)
2. Kim HM, Kim JS, Lee SH, Lee SJ, Lee GP, Kang SS, Cho SH, Cheoi DS. Quantitative analysis of lignans in the fruits of *Acanthopanax* species by HPLC. Food Sci. Biotechnol. 15: 778-780 (2006)
3. Park JE, Soh JR, Rho JO, Cha YS. Effects of cell cultured

Acanthopanax senticosus extract supplementation and swimming exercise on lipid and carnitine profiles in C57BL/6J mice fed a high fat diet. Food Sci. Biotechnol. 16: 894-901 (2007)
4. Bae EA, Yook CS, Oh OJ, Nohara T, Kim DH. Metabolism of chiisanoside from *Acanthopanax divaricatus* var. *albeofructus* by human intestinal bacteria and its relation to some biological activities. Biol. Pharm. Bull. 24: 582-585 (2001)
5. Lee SH, Kim BK, Cho SH, Shin KH. Phytochemical constituents from the fruits of *Acanthopanax sessiliflorus*. Arch. Pharm. Res. 25: 280-284 (2002)
6. Yook CS. Medicinal Herbs of *Acanthopanax* in Asia. Kyeongwon Media, Seoul, Korea. pp. 1-52 (2001)
7. Yoon TJ, Yoo YC, Lee SW, Shin KS, Choi WH, Hwang SH, Ha ES, Jo SK, Kim SH, Park WM. Anti-metastatic activity of *Acanthopanax senticosus* extract and its possible immunological mechanism of action. J. Ethnopharmacol. 93: 247-253 (2004)
8. Oh OJ, Chang SY, Kim TH, Yang KS, Yook CS, Park SY, Nohara T. Constituents of *Acanthopanax divaricatus* var. *albeofructus*. Natural Med. 54: 29-32 (2000)
9. Kim YO, Cho DH, Chung HJ, Kim JH, Chang SY, Yook CS, Yang KS, Oh OJ. Effects of lupane-triterpenoids on mitogen-induced proliferation of lymphocytes. Yakhak Hoeji 43: 208-213 (1999)
10. Zu SM, Yang KS. Anti-lipid peroxidation activity of *Acanthopanax* var. *albeofructus*. Yakhak Hoeji 48: 99-103 (2004)
11. Kim JY, Yang KS. Screening of antioxidant activity of *Acanthopanax* species *in vitro*. Yakhak Hoeji 47: 361-364 (2003)
12. Beutler B, Biron C, Carroll M, Cyster J, Gerard C, Gordon S, Lanier L, Lehrer R, McGhee J, Medzhitov R, Springer T, Yokoyama W. Innate immunity. p. 76. In: Immunobiology. Janeway CA Jr, Travers P, Walport M, Shlomchik MJ (eds). Garland Science Publishing, New York, NY, USA (2005)
13. Oppenheim JJ. Cytokines: Past, present, and future. Int. J. Hematol. 74: 3-8 (2001)
14. Jin CH, Yang U, Kim SH, Ryu JW, Lee JC, Lee DS, Lee TH. The protective effect of chondrotinin from Raja kenojei cartilage on collagen-induced arthritis in DBA/1J mice. Food Sci. Biotechnol. 16: 594-599 (2007)
15. Lucey DR, Clerici M, Shearer GM. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. Clin. Microbiol. Rev. 9: 532-562 (1996)
16. Mossman TR, Sad S. The expanding universe of T-cell subset: Th1, Th2, and more. Immunol. Today 17: 138-146 (1996)
17. Trinchieri G. Interleukin-12: A cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cell type 1 and cytokine lymphocytes. Blood 84: 4008-4027 (1995)
18. Ha ES, Hwang SH, Shin KS, Yu KW, Lee KH, Choi JS, Park WM, Yoon TJ. Anti-metastatic activity of glycoprotein fractionated from *Acanthopanax senticosus*, involvement of NK-cell and macrophage activation. Arch. Pharm. Res. 27: 217-224 (2004)
19. Mackay IR, Rosen FS. Tolerance and autoimmunity. New. Engl. J. Med. 344: 655-664 (2001)
20. O'Garra A, Macatonia SE, Hsieh CS, Murphy KM. Regulatory role of IL4 and other cytokines in T helper cell development in an alpha beta TCR transgenic mouse system. Res. Immunol. 144: 620-625 (1993)
21. Kang JS, Linh PT, Cai XF, Lee JJ, Kim YH. Determination of chiisanoside in *Acanthopanax* species by high performance liquid chromatography. Nat. Prod. Sci. 9: 45-48 (2003)
22. Suto T, Fukuda S, Moriya N, Watanabe Y, Sasaki D, Yoshida Y, Sakata Y. Clinical study of biological response modifiers as maintenance therapy for hepatocellular carcinoma. Cancer Chemoth. Pharm. 33: 145-148 (1994)
23. Schmeda-Hirschmann G, Villasenor-Garcia MM, Lozoya X, Puebla-Perez AM. Immunomodulatory activity of Chilean *Cyttaria* species in mice with L5178Y lymphoma. J. Ethnopharmacol. 77: 253-257 (2001)
24. Saiki I. A *kampo* medicine '*Juzen-taiho-to*'-prevention of malignant progression and metastasis of tumor cells and the mechanism of action. Biol. Pharm. Bull. 23: 677-688 (2000)
25. Ohnishi Y, Fuji H, Hayakawa Y, Sakukawa R, Yamaura T, Sakamoto T, Tsukada K, Fujimaki M, Nunome S, Komatsu Y, Saiki I. Oral administration of a *kampo* (Japanese herbal) medicine *Juzen-taiho-to* inhibits liver metastasis of colon 26-L5 carcinoma cells. Jpn. J. Cancer Res. 89: 206-213 (1998)
26. Albanidou-Farmaki E, Markopoulos AK, Kalogerakou F, Antoniades DZ. Detection, enumeration, and characterization of T helper cells secreting type 1 and type 2 cytokines in patients with recurrent Aphtous stomatitis. Tohoku J. Exp. Med. 212: 101-105 (2007)
27. Mosmann T. T Cell-mediated immunity: The production of armed effector T cells. p. 341. In: Immunobiology. Janeway CA Jr, Travers P, Walport M, Shlomchik MJ (eds). Garland Science Publishing, New York, NY, USA (2005)
28. Cher DJ, Mossman TR. Two types of murine helper T cell clone. Delayed-type hypersensitivity is mediated by Th1 clones. J. Immunol. 138: 3688-3694 (1987)
29. Glimcher LH, Murphy KM. Lineage commitment in the immune system: The T helper lymphocyte grows up. Gene Dev. 14: 1693-1711 (2000)
30. Nishimura T, Ohta A. A critical role for antigen-specific Th1 cells in acute liver injury in mice. J. Immunol. 162: 6503-6509 (1999)
31. Nishimura T, Nakui M, Sato M, Iwakabe K, Kitamura H, Sekimoto M, Ohta A, Koda T, Nishimura S. The critical role of Th1-dominant immunity in tumor immunology. Cancer Chemother. Pharmacol. 46: S52-61 (2000)
32. Strassmann G, Jacob CO, Evans R, Beall D, Fong M. Mechanisms of experimental cancer cachexia. Interaction between mononuclear phagocytes and colon-26 carcinoma and its relevance to IL-6-mediated cancer cachexia. J. Immunol. 148: 3674-3678 (1992)
33. Janeway CA Jr, Travers P, Walport M, Shlomchik MJ. General properties of armed effector T cells. pp. 318-319. In: Immunobiology: The Immune System in Health and Disease. Austin P, Lawrence E (eds). Garland Publishing, New York, NY, USA (2001)