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Immunomodulatory activities of ethanolic extract of *Drynariae Rhizoma*

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骨碎補 ethanol 추출물의 면역 조절 작용에 관한 연구

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목적 : 骨碎補 (*Drynariae Rhizoma*)는 한의학에서 골격계 질환의 치유력을 강화시키는 것으로 알려져 있는데, 항 virus, 항 박테리아, 항 염증 작용이 있는 것으로 보고되고 있다. 질병을 치료하기 위하여 면역 반응을 조절하는 기전에 관하여 오랫동안 많은 관심을 기울여왔는데, 식물에서 추출한 약재들이 면역기능을 조절할 수 있는 가능성에 대하여 광범위하게 연구되었다. 이에 저자는 骨碎補의 ethanol 추출물을 가지고 항 세포성과 면역 조절 기능에 대하여 연구하였다.

방법 : 사람의 혈액단핵구 (PBMC)의 배양은 thymidine법으로 검정하고 nitric oxide (NO) 생성은 mouse macrophage RAW 264.7 세포주를 이용하였으며 IL-2, IFN와 TNF-a 생성은 ELISA 기술로 검정하였다. 세포 증식은 FACScan으로 측정하고 세포 표면항원 CD16, CD25 및 HLA-DR은 FITC/PE 항체로 측정하였다.

결과 : 骨碎補는 mitogen (phytohaemagglutinin; PHA)과 antigen (purified protein derivative; PPD)에 의해 자극받은 human peripheral blood mononuclear cells (PBMCs)의 증식을 억제하였다. 더욱이, 骨碎補는 mouse와 인간에 기원한 여러 세포들의 성장을 억제하였다. 또한, nitric oxide (NO), interleukin-2 (IL-2)와 tumor necrosis factor-α (TNF-α)의 생성을 억제하였다. 한편, human PBMCs에서 intracytoplasmic interferon-γ (IFN-γ)와 cell surface markers인 CD16, HLA-DR의 expression은 骨碎補에 의하여 영향을 받지 않았으나, CD25 expression은 현저히 통제되었다.

결론 : 骨碎補 ethanol 추출물이 *in vitro*에서 항 증식성과 면역 억제작용을 가지고 있다는 가능성을 의미하는 것으로 사료된다.

Key Words: *Drynariae Rhizoma*, cell surface markers, lymphocyte proliferation, PHA, nitric oxide, IL-2, TNF-α, intracellular IFN-γ

Ⅰ. Introduction

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reactions it is named as an immunostimulative drug which primarily implies stimulation of non specific system, i.e. granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Immunosuppression implies mainly to reduce resistance against infections or stress, and may occur on account of environmental or chemotherapeutic factors. Immunostimulation and immunosuppression both need to be tackled in order to regulate the normal immunological functioning. Hence both immunostimulating agents and immunosuppressing agents have their own standing and search for better agents exerting these activities

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is becoming the field of major interest all over the world[1].

Natural adjuvants, synthetic agents, antibody reagents are used as immunosuppressive and immunostimulative agents. But there are major limitation to the general use of these agents such as increased risk of infection and generalized effect throughout the immune system[1]. Traditional Chinese medicine system have suggested means to increase the body's natural resistance to disease. A number of traditional medicinal plants have been claimed to possess immunomodulatory activity[2,3].

An increasing number of individuals are adopting complementary and alternative medicine[4]. The overwhelming majority of patients using complementary and alternative medicines do so to complement conventional care, and not as an alternative to it. Several plants used in traditional medicine systems have been shown to modulate immune response[5]. Various active principles have been isolated and characterized from these plants[6,7]. I am interested to identify clinically useful and safe products from medicinal plants that could modulate immune response and may have future in clinic[5].

The traditional herbal medicines, *Drynariae Rhizoma* (*DR* ; Gu-Sui-Bu in Chinese name] was commonly used to manage disorders of orthopedics and had been claimed to have therapeutic effects on bone healing[8]. Specifically, through tissue culture and isotope tracing, it was found that *DR* injection significantly promoted calcification of the cultivated chick embryo bone primordium, increased alkaline phosphatase activity in the cultivated tissue, and accelerated synthesis of proteoglycan[9]. Liu *et al.* has also shown that *DR* has an antioxidant effect on rat osteoblasts from hydrogen peroxide-induced death and may promote bone recovery under similar pathologic conditions[10]. *DR* should be intensively studied for its possible use in bone diseases. *DR* is also known to be effective for the treatment of inflammation, hyperlipidemia, arteriosclerosis, rheumatism, and gynecological diseases such as osteoporosis and bone resorption in oriental medicine[11,12].

Increasing importance of *DR* has led to the development of technique for its *in vitro* propagation. Thus, this study demonstrates anticellular and immunosuppressive potential of ethanolic extract from *DR*.

II. Materials and methods

1. Cells and reagents

Human T cells (Jurkat), erythroleukemic cells (K562), monocytic cells (THP-1), macrophage cells (U937), mouse liver cells (BNLCL.2), fibroblast cells (L929), and lymphoma cells (EL-4) were grown in RPMI-1640 (Sigma, St. Louis, MO, USA) supplemented with 10% fetal calf serum (FCS; Biological Industries, Kibbutz Beik Haemek, Israel), penicillin (100 U/ml) and streptomycin (100 g/ml). Mouse macrophage cells (RAW 264.7) and human embryonic kidney cells (HEK293) were maintained in Dulbecco's modified eagles medium (DMEM; Sigma) with above supplements. Phytohaemagglutinin (PHA), phorbol 12-myristate 13-acetate (PMA), lipopolysaccharide (LPS) and Greiss reagent (1% sulfanilamide and 0.1% N-[napthyl]ethylenediamine dihydrochloride in 2.5% H_3PO_4) were procured from Sigma. Purified protein derivative (PPD) was a gift from Span Diagnostics, Mumbai, India. ELISA kits for interleukin-2 (IL-2) and tumor necrosis factor-α (TNF-α) were purchased from R&D Systems (Minneapolis, MN, USA). Phycoerythrin (PE)-conjugated anti-human antibodies for intracytoplasmic interferon-γ (IFN-γ), CD16, CD25, flourescein-isothiocyanate

(FITC)-conjugated anti-human CD3, HLA-DR, and intracellular IFN-γ staining kits were obtained from BD-Pharmingen (San Jose, CA, USA).

2. *Drynariae Rhizoma* (*DR*) extracts preparation

DR was kindly supplied by the Oriental Medical Hospital, College of Oriental Medicine, Dongguk University (Kyungju, Korea). The freshly collected *DR* was dried in shade, powdered (200 g), placed in glass percolator and then submerged in ethanol (95%). After standing for 16 h (overnight) at room temperature, the alcoholic extract was drained off. This process of extraction at ambient temperature was repeated four times. The combined alcoholic extract filtered through filter paper and evaporated to dryness under reduced pressure in rotavapour at 45℃ and finally dried in high vacuum furnished viscous extract (42.2 g). The dry ethanolic extract was resuspended in incomplete media for use in cultures.

3. Human PBMC proliferation assay

The lymphocyte proliferation assay was carried out with human peripheral blood mononuclear cells (PBMCs) as described earlier[13]. In brief, cultures were set up in 96-well round bottom plates (Costar, Cambridge, MA, USA), each well containing 0.2 ml of cell suspension (0.5×10^6 cells/ml). The positive stimulant included PHA (2 g/ml) or PPD (20 g/ml). Cells were incubated with or without *DR* either in the presence or absence of these stimulants for a total of 72 or 120 h, and were pulsed with 0.25 Ci [3H]-thymidine/well (925 GBq/mmol [27 mCi=1 GBq], Amersham Pharmacia Biotech, Buckinghamshire, England) per well during the last 18 h of incubation. Thymidine uptake was determined by liquid scintillation counter (LKB, Fullerton, CA, USA). The mean count per minute (CPM) of five replicate well cultures was calculated for each set of cultures. Data have been presented as CPM±S.D. (standard deviation).

4. Proliferation assay with various cell lines

Cell proliferation assay with various cell lines mentioned above was carried out using single cell suspension as described earlier[14]. Five well cultures were set up in 96-well flat bottom plate at a concentration of 0.1×10^6 cells/ml, and assay was carried out for 72 h as described above for PBMCs.

5. Nitric oxide (NO) estimation

NO was measured as nitrite released from mouse macrophage cells, RAW 264.7. Cells were suspended in DMEM supplemented with 10% FCS and plated in 96-well culture plates (25×10^4 cells/ml). Culture was incubated for 48 h at 37℃ in an atmosphere of 5% CO_2 and 95% humidity. Thereafter, 100 ℓ of media from each well was aspirated and replenished with same amount of fresh media, and further incubated for 24 h with desired concentration of extract in the presence or absence of LPS (0.5 μg/ml). NO production in supernatant was measured by microplate assay[15]. To measure nitrite, 100 ℓ supernatant was mixed with an equal volume of the Griess reagent at room temperature for 10 min. The absorbance at 570 nm was determined in a microtiter plate reader (Titertek, Helsinki, Finland). NO estimation was carried out using standard curve plotted against known quantity of sodium nitrite. Result is presented in M concentration obtained from mean OD of triplicate wells of each group.

6. IL-2 and TNF-α production and estimation

IL-2 and TNF-α stimulation and estimation was carried out as described earlier[16]. In brief, a

suspension of human PBMCs (0.5×10^6 cells/ml) was cultured in RPMI-1640 for 18 h in the presence or absence of *DR*. PHA (1.25 μg/ml) and LPS (10 ng/ml) were used as stimulant for IL-2 and TNF-α, respectively. Supernatant samples were harvested and stored at -70℃ until tested. For estimation, ELISA for IL-2/TNF-α was carried out as solid phase enzyme immunoassay based on multiple antibody sandwich principle[17]. The absorbance measured at 450 nm was proportional to the concentration of IL-2/TNF-α present in samples. Standard curve was obtained by plotting known concentrations of IL-2/TNF-α versus absorbance. The cytokine concentrations in experimental samples were determined with standard curve. Results are presented as concentrations in pg/ml.

7. Intracellular staining for IFN-γ

Intracellular IFN-γ staining was carried out as described recently[18]. In brief, whole diluted blood was incubated in polypropylene tubes with 100 ng/ml of PMA and 5 μg/ml of PHA at 37℃ for 8 h with or without *DR*. After 1 h of incubation, 2 μℓ GolgiStop was added and after 8 h of further incubation, cells were exposed to PharMalyse (Becton Dickinson, Palo Alto, CA, USA) to lyse the cells. Then cells were washed in staining buffer and incubated with anti-CD3 for 15 min at room temperature in dark. The cells were washed in staining buffer and fixed/permeabilized with Cytofix/Cytoperm buffer. Again, cells were washed and incubated with PE-conjugated IFN-γ for 30 min in dark and washed once more. The stained cells were assessed by two-color flow cytometric analysis on a FACScan (FACScalibur, Becton Dickinson).

8. Staining for cell surface marker

Healthy volunteer whole blood was incubated overnight with various concentrations of *DR* either alone or in combination with positive stimulants. Cell surface expression for CD16, CD25 and HLA-DR was evaluated by FITC/PE-conjugated monoclonal antibody with a flow cytometer. Two-color analysis was carried out in flow cytometry using a lysed whole blood technique[19]. Background fluorescence was assessed with the appropriate isotype and fluorochrome matched control monoclonal antibody. Analysis was carried out in FACScan using Cell Quest software. PMA/PHA and LPS were used as positive stimulants for CD25 and HLA-DR, respectively.

9. Statistical analysis

The data were analyzed using Sigma Stat. Student's t-test was applied to see statistical significance. The p value < 0.05 was considered to be significant.

III. Results

1. Effect of various concentrations of *Drynariae Rhizoma* on cell viability

To rule out the toxic effects of various concentrations of *Drynariae Rhizoma* (*DR*) used in this study, I tested its effect on viability of human erythroleukemic K562 and monocytic THP-1 cells by trypan blue dye exclusion test. Exposure of cells to *DR* (100 μg/ml) did not show any adverse effect on viability of these cells as compared to untreated controls (Table 1). I have also tested the cell viability with propidium iodide staining using FACScan for confirmation (data not presented).

2. Effect of *DR* on human PBMC proliferation

To investigate the effects of *DR* on mitogenic and antigenic stimulation of T-cell proliferation, PHA

and PPD-stimulated human PBMCs were tested. Human PBMCs were cultured for 72 h in the presence of mitogen (PHA) with or without *DR*. Human PBMCs culture stimulated by PHA was significantly inhibited by *DR* at 5, 10 and 50 μg/ml concentrations (Table 2). Furthermore, PBMCs culture of PPD positive individuals stimulated with PPD *in vitro* was also inhibited significantly by *DR* (Table 2) showing that *DR* extract can inhibit antigen stimulated T-cell proliferation, in addition to mitogenic proliferation.

3. Effect of *DR* on proliferation of various cell lines

To evaluate the effects of *DR* on different cells, experiment was carried out with various cell lines of mouse and human origin. Cells were incubated in the presence or absence of *DR* for 72 h. During last 18 h cells were pulsed with 3H-thymidine. Extract of *DR* significantly inhibited proliferation of different cell lines of human and mouse origin (RAW 264.7, K562, THP-1, U937, HEK293, L929, Jurkat, EL-4 and BNLCL.2) as demonstrated in Fig. 1.

4. Effect of *DR* on NO production

DR was evaluated for its effect on NO production in mouse macrophage cells, RAW 264.7. Ethanolic extract of *DR* significantly inhibited LPS induced

Table 1. Effect of *Drynariae Rhizoma* Extract on Viability of K562 and THP-1 Cells by Trypan Blue Dye Exclusion Test

Group	% Viability of K562 cells			% Viability of THP-1 cells		
	24h	48h	72h	24h	48h	72h
Control	92.2	93.5	93.2	90.3	92.2	91.4
DR (50μg/ml)	92.1	93.2	91.3	89.4	93.3	91.7

Cells were incubated with or without *DR* for different periods, and stained with trypan blue dye. 94.6% of K562 and 92.6% of THP-1 cells were viable at 0 h.

Table 2. Effect of *Drynariae Rhizoma* on Mitogen (PHA)-Stimulated Human PBMCs Proliferation Trypan Blue Dye Exclusion Test

Treatments	Mean CPM±S.D.
Control	1120±132
PHA	51230±4231
DR (5μg/ml)	823±56 (28.4)*
DR (10μg/ml)	811±35 (30.5)*
DR (50μg/ml)	783±42 (36.3)*
DR (5μg/ml) + PHA	18423±1556 (60.5)**
DR (10μg/ml) + PHA	15267±1745 (67.4)**
DR (50μg/ml) + PHA	12453±2123 (74.3)#

Proliferation assay was carried out with human PBMCs. Cells were cultured for a total of 72 h and pulsed with 0.25 uCi [3H]-thymidine during last 18 h. PHA was used at a concentration of 2 μg/ml. Percent inhibition is given in parenthesis. CPM is mean of five wells from one of the three representative experiments. p Value was calculated in comparison to PHA.
* : $p < 0.05$, ** : $p < 0.01$, # : $p < 0.05$.

NO production at 50 g/ml concentration in RAW 264.7 cells when cells were incubated with extract for 24 h ($p<0.05$) (Fig. 2). Inhibition of NO at lower concentrations was statistically insignificant (data not presented).

5. Effect of *DR* on IL-2 and TNF-α production

I was also interested to check the effects of *DR*

Table 3. Effect of *DR* on Antigen (PPD)-Stimulated Human PBMCs Proliferation

Treatments	Mean CPM±S.D.
Control	4021±653
PPD	5250±567**
DR (5μg/ml)	3533±432 (8.5)
DR (10μg/ml)	2836±256 (34.7)**
DR (50μg/ml)	2230±254 (40.6)**
DR (5μg/ml) + PPD	4670±465 (12.4)**
DR (10μg/ml) + PPD	3756±280 (26.8)*
DR (50μg/ml) + PPD	3032±423 (35.2)*

Cells were cultured for a total of 120 h and pulsed with 0.25 uCi [3H]-thymidine during last 18 h. PPD was used at a concentration of 20 μg/ml. Percent inhibition is given in parenthesis. CPM is mean of five wells from one of the three representative experiments. p Value was calculated in comparison to PPD. For PPD group, comparison was made with control.
* : $p<0.05$, ** : $p<0.01$.

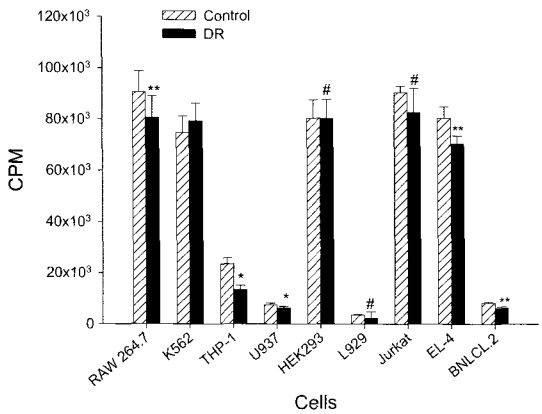

Fig. 1. Effect of *DR* on proliferation of various cell lines. Cultures were set up in 96-well flat bottom plate at a concentration of 0.1×10^6 cells/ml (20×10^3 cells/ well). *DR* at concentration of 50 μg/ml was used in all experiments. Cells were cultured for a total of 72 h and pulsed with 0.5 uCi [^{3}H]-thymidine during last 18 h. Error bars represent standard deviation of the mean values.

* : $p<0.05$, ** : $p<0.01$, # : $p<0.05$.

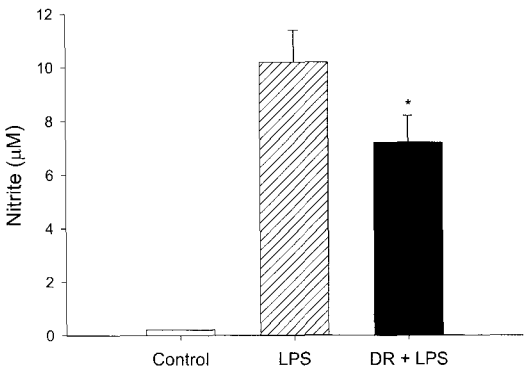

Fig. 2. Effect of *DR* on LPS-stimulated NO production in RAW 264.7 cells. Cells were incubated with extract for 24 h in the presence of LPS. NO was estimated as nitrite in the supernatant. Data of three separate experiments has been pooled. LPS concentration used was 0.5 g/ml. Nitrite concentration is given in μM.

* : $p<0.05$.

on induction of IL-2 and TNF-α. Human PBMCs were used for evaluating the effect of *DR* on the production of IL-2 and TNF-α. *DR* significantly inhibited PHA-stimulated IL-2 production at a concentration of 50 μg/ml as well as LPS-stimulated TNF-α production in human PBMCs when stimulated for 18 h (Fig. 3, Fig. 4).

6. Flow cytometric analysis for intracellular IFN-γ, CD16, CD25 and HLA-DR

To understand the mechanism of immunosuppressive potential of *DR*, intracytoplasmic IFN-γ stimulation and various cell surface marker expressions were investigated. The percentage of CD3+ IFN-γ cells in the peripheral blood was enumerated. Untreated control and *DR*-treated samples did not show significant difference (Table 4). Cells treated with positive stimulant (PMA and PHA) showed 34.6% intracellular staining for IFN-γ while sample treated with *DR* (50 μg/ml) in the presence of PMA/PHA stained 31.3% cells showing no significant inhibition of IFN-γ producing cells.

Dot plot analysis of the lymphocyte-gated population in untreated control showed 13.6% cells stained with anti-CD16 antibody while *DR*-treated cells (50 μg/ml) showed 14.2% of CD16 positive cells (Table 4). In brief, treatment with *DR* did not affect expression of CD16 on cells.

Effect of *DR* extract on expression of CD25 (T-cell activation antigen-Tac) surface molecules was determined using PMA/PHA as positive stimulant. Dot plot analysis of lymphocyte-gated population showed 13.0% of CD25 positive cells in untreated control. PMA and PHA treatment stained 27.4% cells with anti-CD25 antibody. *DR* combination with PMA and PHA stained only 18.5% for CD25 expressing cells (Table 4). This observation shows that treatment of *DR* extract down regulates CD25 expression stimulated with PMA and PHA. This may be relevant since *DR* significantly inhibits T-cell proliferation.

Dot plot analysis for activation marker HLA-DR

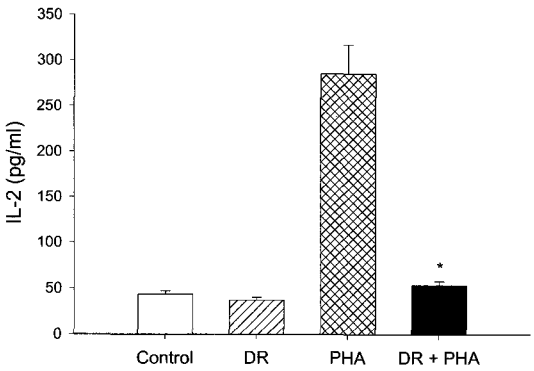

Fig. 3. Effect of *DR* on IL-2-production in human PBMCs. Human PBMCs (0.5×10^6 cells/ml) was cultured in RPMI-1640 for 18 h in the presence or absence of 50 μg/ml of *DR*. PHA (1.25 μg/ml) was used as positive control to stimulate IL-2, respectively. Error bars represent standard deviation of the mean values.

* : $p<0.05$, ** : $p<0.01$.

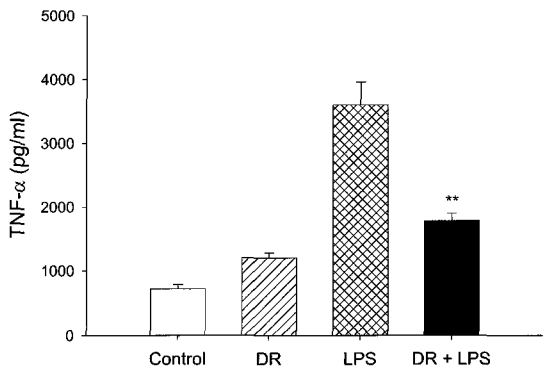

Fig. 4. Effect of *DR* on TNF-α production in human PBMCs. Human PBMCs (0.5×10^6 cells/ml) was cultured in RPMI-1640 for 18 h in the presence or absence of 50 μg/ml of *DR*. LPS (10 ng/ml) was used as positive control to stimulate TNF-α, respectively. Error bars represent standard deviation of the mean values.

** : $p<0.01$.

Table 4. Effect of *DR* on Intracellular IFN-γ and Other Cell Surface Markers

Parameters cells	Percentage of positive cells		
	Control	Positive stimulant	*DR* (50 μg/ml)
Intracellular IFN-γ	10.9	34.6	31.3
CD16	13.6	ND	14.2
CD25	13.0	27.4	18.5
HLA-DR	14.2	15.6	14.0

Whole blood obtained from healthy individuals was diluted in RPMI-1640 and incubated with various stimulants. PMA (100 ng/ml) and PHA (5 μg/ml) were used as positive stimulant for intracellular IFN-γ and CD25 staining. LPS (1 μg/ml) was used for activation of HLA-DR as positive control. *DR* was used at 50 μg/ml in the presence of positive stimulants. The stained cells were assessed by two-color flow cytometric analysis. Table represent percentage cells in lymphocyte-gated population. ND=Not done.

showed 14.2% of cells in the control sample while 14.0% cells got stained in *DR* (50 g/ml)-treated samples (Table 4). In brief, *DR* did not affect HLA-DR expression.

Ⅳ. Discussion

There are several medicinal plants having well-documented immunomodulatory and antiproliferative properties[5,20,21]. Modulation of immune response by medicinal plant products as a possible therapeutic measure has become a subject of scientific investigation recently.

Traditional Chinese medicines may offer advantages over synthetic agent medication for a long time[22]. Initial studies showed that herbal medicines that have traditionally been effective for the gynecological diseases[23,24] may also be administered for the prevention of osteoporosis. One of the traditional Chinese medicine, *Drynariae Rhizoma* (*DR* : Gu-Sui-Bu in Chinese name) has been alleged to have therapeutic effects on bone healing[8,10]. Liu *et al.*[8,10] reported that *DR* is not only non-cytotoxic but also has an anti-oxidative effect on osteoblasts[23]. Administration of *DR* can accelerate the speed of intracellular ALP synthesis by the bone cells. Since a major feature of bone is its continuous remodeling, the molecular constituents of bone are closely related to this process. They demonstrated that *DR* has potential effects on the bone cells culture and that one of the major effects of *DR* on the bone cells is probably mediated by its effect on the osteclasts attachment.

I have observed that ethanolic extract of *DR* at 5, 10 and 50 μg/ml concentrations has significant antiproliferative action on mitogen (PHA) and antigen (PPD)-stimulated human PBMCs. *DR* also inhibited proliferation of several other cell lines (monocytic, lymhoblastoid, fibroblast, erythroleukemic) of mouse and human origin demonstrating that the effect is not cell specific. I have checked the effect of *DR* on viability of these cells and observed that up to 50 μg/ml concentration of this product has no adverse effect on cell viability when exposed for 72 h (Table 1). There are several plant products showing anticellular activity in different cells[25-29].

NO has diverse physiological roles and contributes to the immune defense against pathogens[30]. Ethanolic extract of *DR* significantly inhibited LPS-induced NO production in mouse macrophage cells, RAW264.7 (Fig. 2). Its ethanolic extract was found to be more effective than aqueous extract.

Effect of *DR* on IL-2 production evaluated in the

culture supernatant showed an inhibition at 50 μg/ml concentration (Fig. 3). Inhibition in the level of TNF-α production at the same concentration was also observed (Fig. 4). Human PBMCs were used for the stimulation of IL-2 and TNF-α with appropriate positive stimulants. TNF-α is believed to be involved in the pathophysiology of microbial infections and their sequelae[31]. The primary mechanism of anti-inflammatory actions of some other plant extracts and their role in immunomodulation has been shown to be through inhibition of TNF-α produced, and scavenging of free radical production[32]. Thus, *DR* may also be of therapeutic importance since it inhibits NO production.

Results of flow cytometry analysis show that *DR* has no significant effect on intracellular IFN-γ production, CD16 and HLA-DR expression. Monocyte activation as determined by cell surface expression of HLA-DR in peripheral blood lymphocyte[33] and human tumor derived cells[34] by bacterial LPS, recombinant IFN-γ and phorbol ester have been reported earlier. Failure of *DR* to inhibit these cell surface marker expressions suggests that its inhibitory effect on other parameters may be through different pathway.

Treatment of human PBMCs with 50 μg/ml of *DR* inhibited expression of CD25 surface molecule induced by PMA and PHA. CD25 is a unique subunit of the high affinity IL-2 receptor (IL-2R). Resting T cells express few IL-2R. However, when activated, the expression of IL-2R rapidly increases. Dot plot analysis of lymphocyte-gated population exhibited 14.2% of CD25 positive cells in untreated sample. PMA and PHA treatment stained 26.60% cells with anti-CD25 antibody while *DR* in combination with PMA and PHA stained only 18.5% cells. This observation supports the result of mitogen and antigen-stimulated lymphocyte proliferation inhibition by A. calamus. *DR* prevents cell activation by PHA and PMA.

In brief, our present study demonstrates antiproliferative and immunosuppressive potential of ethanolic extract of *DR*. We used the ethanolic extract of *DR* to evaluate its immunomodulatory potential. In-depth investigation with various fractions is needed to identify the active principle of this extract and to elucidate the mechanism of action. Currently, we are in the process of fractionating ethanolic extract to identify active component(s).

Ⅴ. Abstracts

In the traditional Chinese medicine, *Drynariae Rhizoma* (*DR*) has been reported as a good enhancer for bone healing. *DR*, a plant widely used in the traditional medicinal systems of Korea, has been reported to possess antiviral, antibacterial and anti-inflammatory activities. Modulation of immune response to alleviate disease has been of interest for a long time. Plant extracts have been widely investigated for possible immunomodulatory properties. Thus, I have evaluated the anticellular and immunomodulatory properties of ethanolic extract of *DR*.

DR extract inhibited proliferation of mitogen (phytohaemagglutinin; PHA) and antigen (purified protein derivative; PPD)-stimulated human peripheral blood mononuclear cells (PBMCs). In addition, *DR* inhibited growth of several cell lines of mouse and human origin. It also inhibited production of nitric oxide (NO), interleukin-2 (IL-2) and tumor necrosis factor-α (TNF-α). Intracytoplasmic interferon-γ (IFN-γ) and expression of cell surface markers, CD16 and HLA-DR, on

human PBMC, were not affected on treatment with *DR* but CD25 expression was down regulated. This study demonstrates the antiproliferative and immunosuppressive potential of ethanolic extract of *DR in vitro*.

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