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Effect of Ginseng Saponins on Phagocytosis of Feline Peripheral Blood Phagocytes

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고양이 말초혈액 탐식세포의 탐식능에 있어서 인삼 사포닌의 효과

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초 록 : 고양이 말초혈액 탐식세포(단핵구세포(MNC) 및 다형핵백혈구(PMNC))의 탐식능에 있어서 인삼 saponin(ginseng total saponin(GTS), ginseng PT saponin(GPT) 및 ginseng PD saponin(GPD))의 면역증강 효과를 flow cytometry를 이용하여 분석하였다. 인삼 saponins을 직접 첨가하여 배양한 MNC 및 PMNC에서는 탐식증강 효과가 나타나지 않았다. 각각의 인삼 saponin을 첨가하여 배양한 PMNC 및 MNC 배양상층액의 존재하에 PMNC 및 MNC의 탐식능을 검토한 결과, MNC의 탐식능은 GPT 첨가 PMNC 배양상층액과 GTS 및 GPT 첨가 MNC 배양상층액의 존재하에서 약간의 탐식증강 효과를 보였다. PMNC 탐식능의 경우에는 GPD 첨가 PMNC 배양상층액에서 미약한 탐식증강 효과가 나타났으나, 각각의 인삼 saponin 첨가 MNC 배양상층액 존재하에서는 모두 현저한 탐식증강 효과를 나타내었다. 이상의 결과로부터 고양이 말초혈액 탐식세포의 탐식증강 효과는 인삼 saponin의 직접적인 작용보다는 인삼 saponin에 의해 활성화된 단핵구세포에서 분비되는 가용성물질에 의해 단핵구세포보다는 다형핵백혈구에서 현저하게 탐식효과가 증강되는 것으로 판단되었다.

Key words : cat, phagocytosis, ginseng saponins, mononuclear cells, polymorphonuclear cells

Introduction

Phagocytes including monocytes-macrophages and neutrophils play important regulatory and functional roles in the immune system[21]. Monocytes-macrophages release many soluble factors associated with host defense and inflammation. Phagocytosis is one of the representative functions of monocytes-macrophages and neutrophils. In addition to their phagocytic properties, monocytes-macrophages are able to synthesize a number of biologically important compounds including several cytokines[20]. Phagocytosis by macrophages is enhanced by platelet activating factor (PAF) in allergic and inflammatory reactions[11]. A variety of biological response-modifiers (BMR) has been reported to have immunomodulating effects in the host in regard to resistance to infectious organisms[2,15,22]. Beta-endorphin[10], dynorphin A[9] and tuftsin (Thr-Lys-Pro-Arg)[14] were found to stimulate phagocytosis. Chicken egg white derivatives (EWD), an active egg white product (AEWP) and chicken egg white-derived immunoactive peptides (EF203), which are supposed to stimulate phagocytic functions, have been also demonstrated to enhance non-specific immunity in mice, piglet and cattle[2,3,13].

Ginseng saponins also increase non-specific resistance of organism and strengthen the defense mechanism of the entire body[4]. Ginseng saponins are dammarane-type triterpenoid glycosides whose agly-

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cons such as panaxatriol and panaxadiol are bonded with glucose, rhamnose, xylose and arabinose[12]. Ginsenosides show many pharmacological properties in various organ such as brain, immune system, cardiovascular system, respiratory system, and endocrine system. Physiological responses of these saponins are diverse or sometimes two directional, e.g., stimulant action of ginsenoside Rg1 and depressant action of Rb1 in central nervous system, increasing action of ginsenoside Rg1 and decreasing action of Rb1 in blood pressure[23].

However, despite the advance of the biological and pharmacological study for ginseng saponins, little is known about the effect of ginseng saponins on the phagocytosis of peripheral blood phagocytes. In current study, the *in vitro* immunostimulating effects of GTS, GPT and GPD on phagocytic responses of peripheral blood mononuclear cells (MNC) as well as peripheral blood polymorphonuclear cells (PMNC) from feline peripheral blood were examined, and a possible involvement of soluble products, phagocytosis-promoting factors, in the enhancement of the phagocytic responses of MNC/PMNC by culture supernatant from MNC exposed to ginseng saponins was described.

Materials and Methods

Laboratory animals

Twenty-four healthy cats, average 1.2-year-old, were housed at animal cage. All cats utilized in this study were free from FeLV infection as examined by an ELISA using a Leukassay F kit (Pitman Moore, NJ, USA).

Reagents

Ginseng total saponin (GTS), PT saponin (GPT) and PD saponin (GPD) were kindly provided by the Korea Ginseng and Tobacco Research Institute (Taejeon, Korea). FITC-labelled latex (latex beads; 2.0 μm) was purchased (Polyscience, Inc., PA, USA).

MNC and PMNC isolation

Peripheral blood was collected in heparinized tubes by jugular venipuncture. Blood diluted 1:1 in phosphate-buffered saline (PBS) at pH 7.6 was layed on the equal volume of Lymphoprep (specific gravity, 1.077; Nycomed Pharma As, Oslo, Norway) and centrifuged at 400×g for 40 min at room temperature. The cells in interface between PBS plus plasma and Lymphoprep were obtained and subjected to 0.83% NH_4Cl in Tris-base buffer (pH 7.6) containing 1% bovine serum albumin (BSA; Sigma, MO, USA) for 5 min at 37°C. The resulting MNC was washed 3 times with PBS. PMNC was obtained from layer of erythrocyte sediment containing PMNC. One ml of pellet of erythrocyte sediment was mixed with 10 ml of 1.5% dextran (molecular weight, 200,000; Wako, Osaka, Japan) in PBS and allowed to sediment for 60 min. The floating cells were collected and centrifuged at 400×g for 5 min. The residual erythrocytes were removed by treatment with 0.83% NH_4Cl solution and washed 3 times with PBS. The purity of neutrophils in PMNC preparations was greater than 96% when determined by cytospin smear and Giemsa stain. The MNC was consisted of approximately 90% lymphocytes and 10% monocytes by modified Wright and Giemsa stain. Cell viabilities of both PMNC and MNC determined by trypan blue dye exclusion always exceeded 98%.

Culture supernatants

Isolated PMNC and MNC at a density of 5×10^6 cells/ml were cultured in a well of a 24-multiwell plate (Falcon 3047, Becton Dickinson Labware, NJ, USA) for 24 hr in RPMI 1640 (Sigma, USA) supplemented with 2 mM L-glutamine, 0.02 mg/ml of gentamicin, and 5% FBS (Gibco, MD, USA). The cells were cultured with GTS (50 μg/ml), GPT (50 μg/ml) and GPD (200 μg/ml) for 24 hr, respectively. All supernatants of cultured PMNC and MNC were collected by centrifugation (5,000×g for 30 min), filtered with 0.45 μm-pore size membrane filter (Millipore, MA, USA) and stored below -70°C until use.

Phagocytosis assay

The phagocytic activity of PMNC and MNC was determined as described previously[8]. The cells at a density of 1×10^6 cells/ml were incubated for 10 hr at 37°C in a 5% CO_2-humidified air atmosphere in a

well of a 24-multiwell plate with either ginseng saponins at different concentrations ranging from 10 to 100 μg/ml or each culture supernatant from MNC and PMNC exposed to ginseng saponins with various concentrations. The control cells were treated with the equal volume of PBS. The cultures were thereafter supplemented with 30 μl of 1×10^9 particles/ml of FITC-labelled latex for the final 1 hr. The cultured cells were harvested gently by slow pipetting, centrifuged at $2,000\times g$ for 1 min, and washed three times with PBS containing 3 mM EDTA-2Na (Wako Pure Chemical Industries, LTD, Tokyo, Japan). The phagocytized latex cells per total 5,000 cells were immediately estimated by a flow cytometry (FACS Calibur, Becton Dickinson Immunocytometry Systems, USA). Positive control was determined by addition of FITC-labelled latex alone. The experiments of one item were repeated in 3 cats. Results were expressed as percentages of either absolute phagocytic activities or relative activities, i.e. percentages of latex-phagocytized cells in the stimulated cells minus percentages of latex-phagocytized cells in the unstimulated cells.

The data expressed as a representative profile of flow cytometry in 3 cats.

Results

Direct effect of ginseng saponins on feline MNC and PMNC phagocytosis

To examine the direct effect of ginseng saponins on phagocytic activity of feline peripheral blood phagocytes, freshly isolated MNC and PMNC were cultured with GTS, GPT and GPD at different concentrations ranging from 10 to 100 μg/ml for 10 hr. The phagocytic activities of MNC (Fig 1) and PMNC (Fig 2) were not augmented by exposure to GTS, GPT and GPD as compared with those in cells treated without ginseng saponins.

Phagocytic response of feline MNC and PMNC in culture supernatant from PMNC exposed to ginseng saponins

To determine whether phagocytic activity of MNC

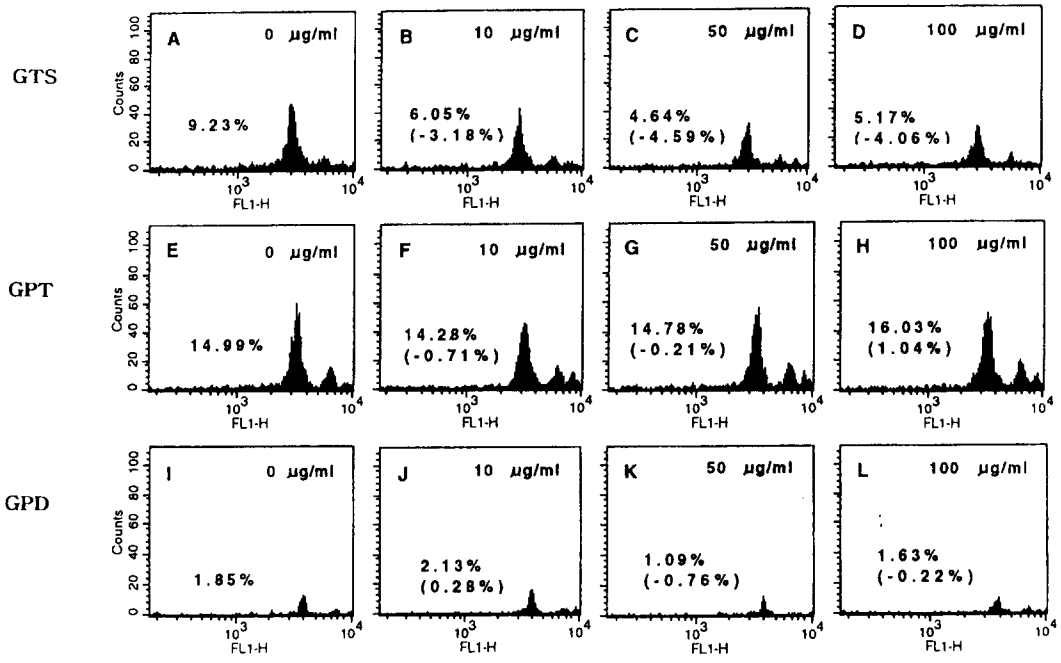

Fig 1. Profiles of phagocytized cells in feline MNC exposed to different concentrations of 10 to 100 μg/ml of GTS, GPT and GPD. Numbers in parenthesis indicate net phagocytic activity calculated by reducing the value of unstimulated cells from that of each stimulated cell.

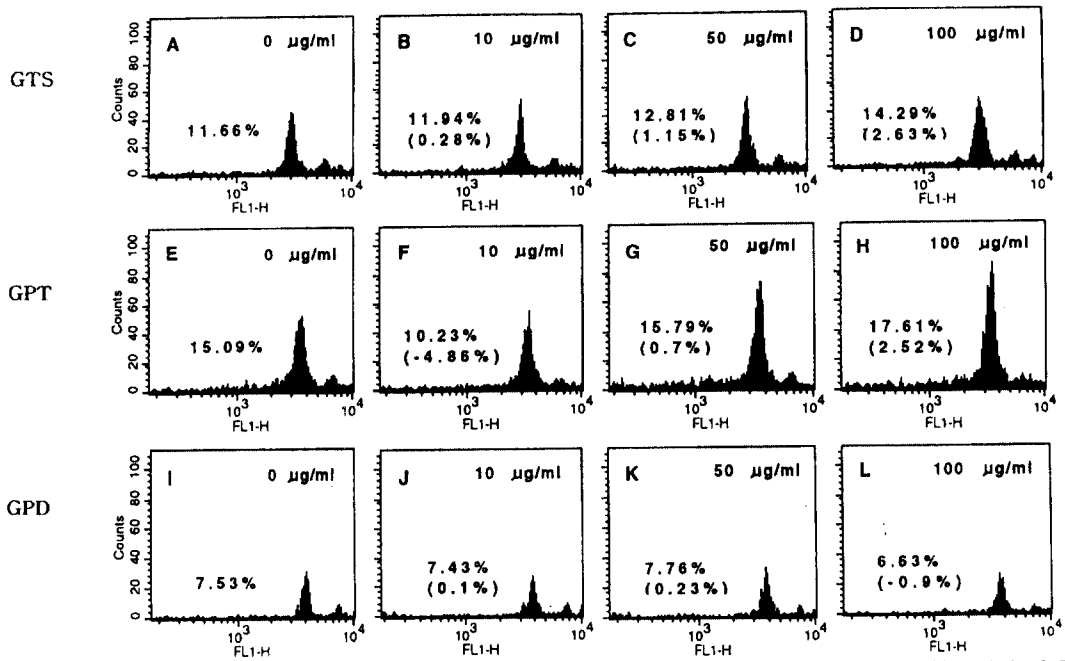

Fig 2. Profiles of phagocytized cells in feline PMNC exposed to different concentrations of 10 to 100 μg/ml of GTS, GPT and GPD. Numbers in parenthesis indicate net phagocytic activity calculated by reducing the value of unstimulated cells from that of each stimulated cell.

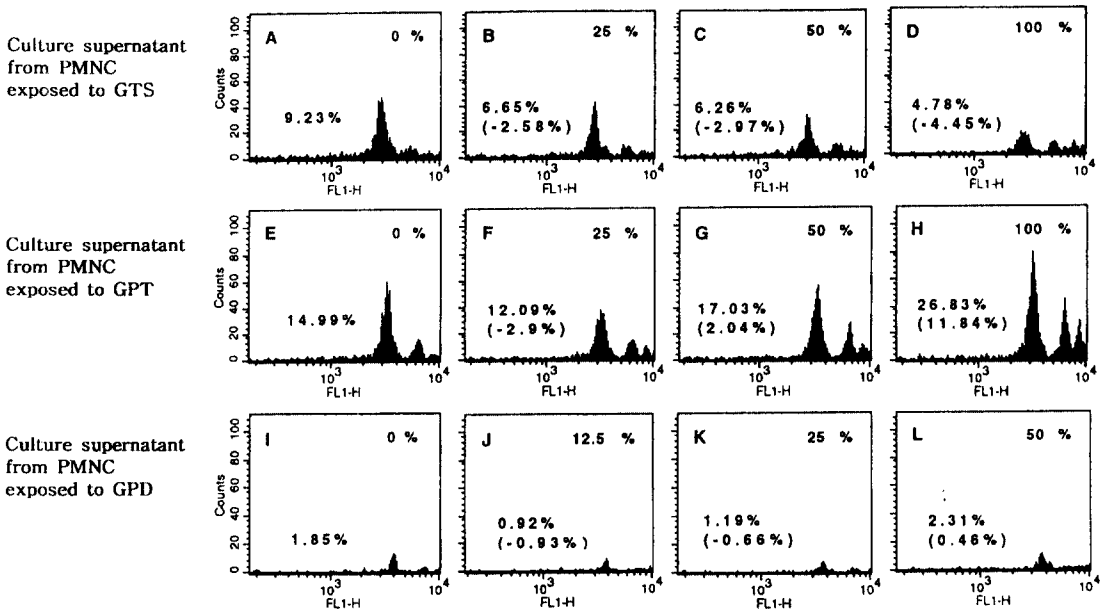

Fig 3. Profiles of phagocytized cells in feline MNC to culture supernatant (12.5 to 100%) from PMNC exposed to GTS (50 μg/ml), GPT (50 μg/ml) and GPD (200 μg/ml). Numbers in parenthesis indicate net phagocytic activity calculated by reducing the value of unstimulated cells from that of each stimulated cell.

and PMNC could be enhanced by addition of culture supernatant from PMNC (5×10^6 cells/ml) exposed to GTS (50 μg/ml), GPT (50 μg/ml) and GPD (200 μg/ml) for 24 hr, freshly prepared MNC and PMNC

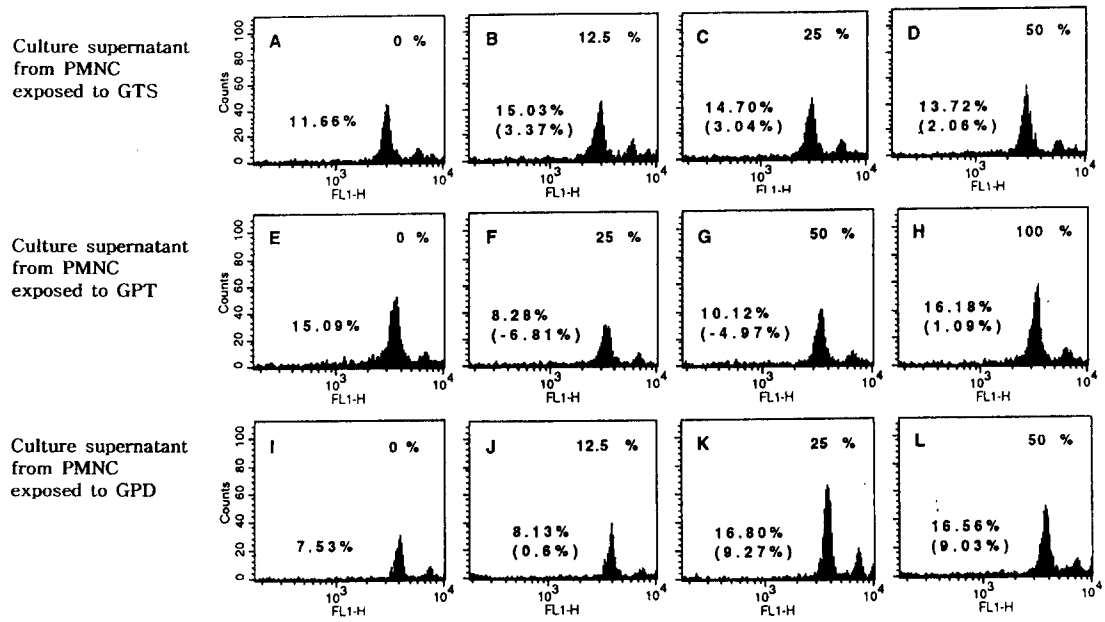

Fig 4. Profiles of phagocytized cells in feline PMNC to culture supernatant (12.5 to 100%) from PMNC exposed to GTS (50 μg/ml), GPT (50 μg/ml) and GPD (200 μg/ml). Numbers in parenthesis indicate net phagocytic activity calculated by reducing the value of unstimulated cells from that of each stimulated cell.

were followed by treatment of culture supernatant from PMNC for 10 hr. The supplement of culture supernatant (100%) from PMNC exposed to GPT for MNC increased about 11% as compared with untreated MNC. Whereas culture supernatants from PMNC exposed to GTS and GPD did not showed any enhancement of phagocytosis (Fig 3). The phagocytosis of PMNC in culture supernatant (25% and 50%, respectively) from PMNC exposed to GPD was enhanced about 9% when compared to that of untreated PMNC (Fig 4). But the supplememt of culture supernatants from PMNC exposed to GTS and GPT tested failed to increase the phagocytosis of PMNC.

Phagocytic response of feline MNC and PMNC in culture supernatant from MNC exposed to ginseng saponins

Phagocytic response of feline MNC and PMNC in culture supernatant from MNC exposed to ginseng saponins was also examined in the same manner. The phagocytic activity of MNC in culture supernatant (25%) from MNC exposed to GTS was enhanced about 9% as compared with that of untreated cells. The activity of MNC in culture supernatant (100%) from MNC exposed to GPT but not GPD was also enhanced about 15% (Fig 5).

However, the phagocytic activity of PMNC in culture supernatant from MNC exposed to ginseng saponins was enhanced remarkably. This enhancement was a dose-dependent manner. As shown in Fig 6, PMNC in culture supernatant (100%) from MNC exposed to GTS, GPT and GPD exhibited strong phagocytoses and their phagocytic activities were enhanced about 54%, 49% and 24% when compared to those of unstimulated PMNC, respectively.

Discussion

The results demonstrated that ginseng saponins have an enhancing effect on phagocytosis of feline PMNC. Culture supernatant of MNC but not PMNC exposed to GTS, GPT and GPD was capable of enhancing the phagocytic activity of feline PMNC which was consist of approximately 96% neutrophils. However, the culture supernatant of PMNC exposed to these saponins had a weak or negligible activity

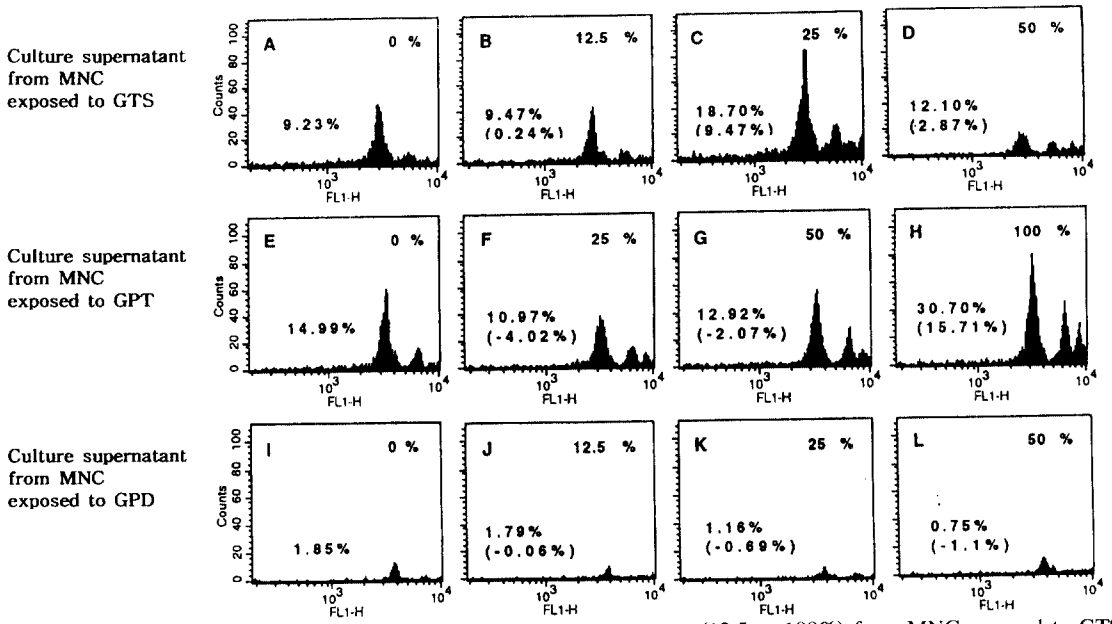

Fig 5. Profiles of phagocytized cells in feline MNC to culture supernatant (12.5 to 100%) from MNC exposed to GTS (50 μg/ml), GPT (50 μg/ml) and GPD (200 μg/ml). Numbers in parenthesis indicate net phagocytic activity calculated by reducing the value of unstimulated cells from that of each stimulated cell.

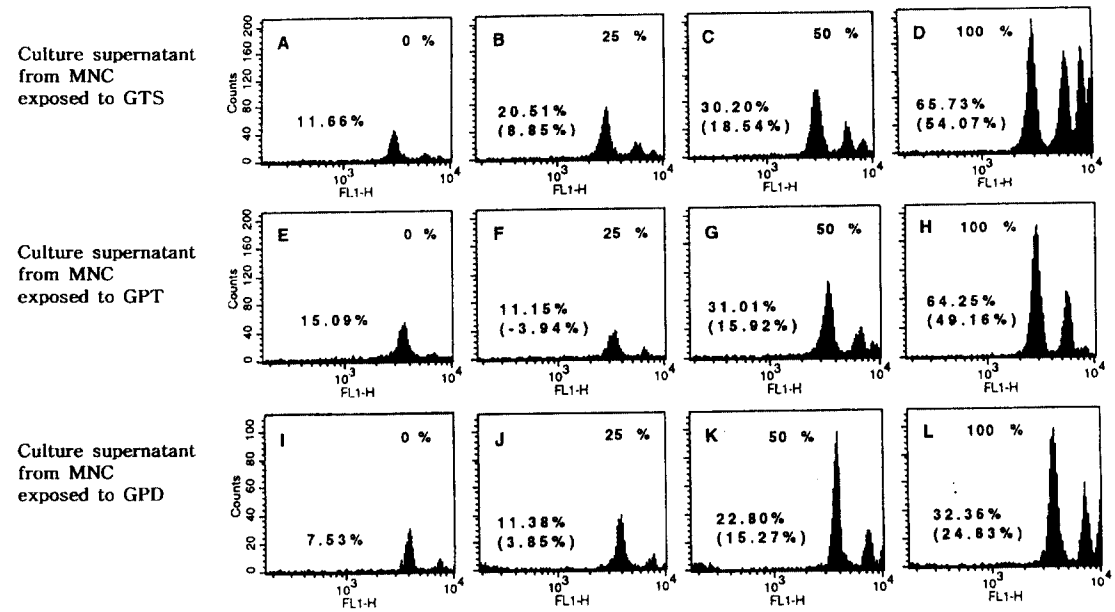

Fig 6. Profiles of phagocytized cells in feline PMNC to culture supernatant (25 to 100%) from MNC exposed to GTS (50 μg/ml), GPT (50 μg/ml) and GPD (200 μg/ml). Numbers in parenthesis indicate net phagocytic activity calculated by reducing the value of unstimulated cells from that of each stimulated cell.

on phagocytosis of MNC and PMNC. Also, direct treatment of ginseng saponins to phagocytes showed no effect on phagocytosis of MNC and PMNC. These findings support that antigenic components of ginseng saponins may be not responsible for the phagocytosis of MNC and PMNC and that MNC ex-

posed to ginseng saponins may secrete the soluble products that can activate themselves or other cells. Thus, these results are strongly suggested that the enhancing effect on phagocytosis of feline peripheral blood neutrophils is primarily mediated by soluble factors from MNC exposed to active components of ginseng saponins. It is also assumed that antigenic components of ginseng saponins act on MNC to produce active soluble products which are involved in the enhancement of PMNC phagocytosis.

GPT at higher concentration of 200 to 400 μg/ml exhibited a low viability of cells. Thus, GTS and GPT was used at concentration of 50 μg/ml showing no cytotoxic effect by dot plot profile in flowcytometry and high cell viablity by trypan blue exclusion (data not shown). Whereas, it is of great interest that the activity of PMNC in culture supernatant from MNC exposed to GTS and GPT was higher than that of GPD. But the concentrations of GTS and GPT used here to prepare the culture supernatant of MNC were lower than those of GPD. These findings indicate that the magnitude in the enhancement of PMNC phagocytosis in culture supernatant of MNC is dependent on the kinds of ginseng saponins, implying the involvement of different humoral substances in the enhancement of PMNC phagocytosis. It is, therefore, suggested that the phagocytic activity by soluble products released from MNC exposed to GPT is more active than those of GPD. On the other hand, the phagocytic activity of MNC to GTS and GPT was lower than that of PMNC. This low responsiveness on phagocytosis of MNC may be, in part, associated with fractions in MNC populations. Because the fractions of MNC isolated from peripheral blood were composed of both approximately 10% monocytes and 90% lymphocytes.

The soluble products of activated monocyte-macrophages and lymphocytes seemed to play important roles in the phagocytosis of phagocytes. These soluble products have been also reported to be involved in the modulation of neutrophil functions including adherence, migration[5], respiratory burst[6], lysosomal enzyme release, and cell surface receptor expression[1,5,7,16-19,28]. Thus, the ability of MNC to release the soluble factors and stimulate the phagocytosis of phagocytes may potentially be implicated in the regulation of cellular infiltration at sites of inflammation or tumor growth. This fact is important in the immuno-compromised host. In summary, the results of the present study suggest that GTS, GPT and GPD stimulate MNC to elaborate soluble products, which may be an important mechanism for the enhancement of phagocytosis.

Conclusion

The objective of this study is to examine the immunostimulating effect of GTS, GPT and GPD on phagocytosis of feline MNC as well as PMNC. The phagocytic activities of MNC and PMNC were analyzed by a flow cytometry system. These ginseng saponins did not show any direct effect on phagocytic response of PMNC and MNC. However, the phagocytic activity of MNC was slightly enhanced by culture supernatants from PMNC exposed to GPT and from MNC exposed to GTS and GPT. Similarly, the phagocytic activity of PMNC was slightly enhanced by culture supernatant from PMNC exposed to GPD. However, the phagocytic activity of PMNC was remarkably enhanced by culture supernatants from MNC exposed to GTS, GPT and GPD. It is, therefore, suggested that the enhanced phagocytic activity of feline PMNC could be mainly mediated by soluble product(s) released from MNC exposed to active components of ginseng saponins.

References

1. Aggarwal BB, Pocsik E. Cytokines: from clone to clinic. Arch Biochem Biophys 1992; 292: 335-359.
2. Araki S, Kagaya K, Kitoh K, Kimura M, Fukazawa Y. Enhancement of resistance to *Escherichia coli* infection in mice by dihydroheptaprenol, a synthetic polyprenol derivative. Infect Immun 1987; 55: 2164-2170.
3. Araki S, Kimura M, Suzuki M, Fujimoto M. Effect of active egg white product on neutrophil function in weaning piglets. J Vet Med Sci 1992; 55: 899-900.
4. Brekhman II, Dardymov IV. New substances of plant origin which increase nonspecific resistance. Am Rev Pharmac 1969; 9: 419-430.

5. Detmers PA, Powell DE, Waltz A, Clark IL, Baggiolini M, Cohn ZA. Differential effects of neutrophil-activating peptide 1/IL-8 and its homologoues on leukocyte adhesion and phagocytosis. J Immunol 1992; 147: 4211-4217.
6. Dewald B, Thelen M, Wymann MP, Baggiolini M. Staurosporine inhibits the respiratory burst and induces exocytosis in human neutrophils. Biochem J 1989; 264: 879-884.
7. Ferrante A, Coffey RG. Activation on neutrophils by interleukins-1 and -2 and tumor necrosis factors. *In*: Granulocyte responses to cytokines, New York: Marcel Dekker. 1992.
8. Hersh EM, Friedman H, Klein TW. Yamaguchi H. Immunomodulatory drugs of relevance to the management of microbial infections, Advences in experimental medicine and biology, vol. 319, Plenum Press, New York. (1992)
9. Ichinose M, Asai M, Sawada M. Enhancement of phagocytosis by dynorphin A in mouse peritoneal macrophages. Journal of Neuroimmunology 1995; 60: 37-43.
10. Ichinose M, Asai M, Sawada M. beta-endorphin enhances phagocytosis of latex particles in mouse peritoneal macrophages. Scandinavian Journal of Immunology 1995; 42: 311-316.
11. Ichinose M, Hara N, Sawada M, Maeno T. A flow cytometric assay reveals an enhancement of phagocytosis by platelet activating factor in murine peritoneal macrophages. Cell Immunol 1994; 156: 508-518.
12. Liu CX, Xiao PG. Recent advances on ginseng reserch in China. J Ethnopharmacol 1992; 36: 27-38.
13. Nakagawa J, Osame S, Ichijyo S, Araki S, Kimura M. Effect of active egg white product on neutrophil function in calves. J Vet Med Sci 1993; 55: 259-263.
14. Nishioka K, Wagle JR, Rodriguez T Jr., Maeta M, Kubo S, Dessens SE. Studies of human granulocyte phagocytosis stimulation by tuftsin. Journal of Surgical Research 1994; 56: 94-101.
15. Ozaki Y, Ohashi T, Minami A, Nakamura S. Enhanced resistance of mice to bacterial infection induced by recombinant human interleukin-1α. Infec Immun 1987; 55: 1436-1440.
16. Quinn PJ . Mechanisms of action of some immunomodulators used in veterinary medicine. In: Immunomodulation in domestic food animals. Ad Vet Sci Comp Med, London: Academic press. 1990
17. Roney CS, Rossi CR, Smith PC, Lauerman LC, Spano JS, Hanrahan LA, William JC. Effect of human leukocyte a interferon on prevention of infectious bovine rhinotracheitis virus infection of cattle. Am J Vet Res 1985; 46: 1251-1255.
18. Roth JA, Abruzzini AF, Frank DE. Influence of recombinant human interleukin 2 adminstration on lymphocyte and neutrophil function in clinically normal and dexamethazone-treated cattle. Am J Vet Res 1990; 51: 546-549.
19. Schultz RM, Kleinshmidt NJ. Functional identity between γ murine interferon and macrophage activating factor. Nature(Lond.) 1983; 305: 239-240.
20. Turgeon ML. Immunology and Serology in Laboratory Medicine, 2nd ed. St, Louis, Missouri: Mosby-Year Book. 1996
21. Unanue ER, Allen PM. The basis for the immunoregulatory role of macrophages and other acrssory cells. Science 1982; 236: 551-557.
22. Wu-hsieh BB, Howard DH. Inhibition of the intracelluar growth of *Histoplasma capsulatum* by recombinant murine gamma interferon. Infec Immun 1987; 55: 1014-1016.
23. 박명규. 인삼효능 · 제품연구결과요약집 제1집. 대전: 한국인삼연초연구원. 1993.