한국임상수의학회지 15(1) : 110-115 (1998)
Korean J. Vet Clin Med. 15(1) : 110-115 (1998)

Interleukin 8-like Chemotactic Activity in the Culture Supernatant from Feline Peripheral Blood Mononuclear Cells Exposed to Ginseng PD Saponin

Mhan-pyo Yang[1] and Se-hyung Park
College of Veterinary Medicine, Chungbuk National University, Cheongju, 361-763, Korea

인삼 PD 사포닌으로 배양한 고양이 말초혈액 단핵구세포 배양상층액중의 interleukin 8 樣 유주활성

양만표[1] · 박세형
충북대학교 수의과대학

초 록 : 인삼 PD saponin(GPD)으로 배양한 고양이 말초혈액 단핵구세포(MNC) 배양상층액에서 말초혈액 다형핵백혈구(PMNC)에 대한 interleukin(IL) 8 樣 유주활성에 관하여 검토하였다. PMNC의 유주활성은 Boyden chamber 변법으로 측정하였다. GPD를 첨가하여 배양한 MNC 배양상층액중에는 PMNC에 대한 유주활성이 인정되었다. PMNC에 대하여 GPD로 배양한 MNC 배양상층액중에 존재하는 유주활성이 IL 8 樣 물질인지를 알아보기 위해 human recombinant IL 8을 이용하여 고양이 PMNC에 대해 유주활성을 측정한 결과, GPD로 배양한 MNC 배양상층액의 경우와 동등한 활성이 나타났다. Human IL 8 mAb를 사용하여 GPD로 배양한 MNC 배양상층액중의 유주활성에 대한 중화반응을 살펴본 결과, GPD로 배양한 MNC 배양상층액 및 human IL 8에 의해 증가되었던 PMNC의 유주활성은 IL 8 mAb의 첨가농도가 증가함에 따라 활성이 완전히 억제되었다. 또한 GPD로 배양한 고양이 MNC 배양상층액중의 유주활성은 열처리(4, 20, 37, 60 및 100°C) 및 산(pH 3.0)과 알카리(pH 9.0)처리에도 안정성을 보여 human IL 8의 물리화학적 성상과 매우 유사하였다. 따라서 GPD로 배양한 MNC 배양상층액중에 존재하는 고양이 PMNC에 대한 유주활성은 feline IL 8 樣 물질임을 강하게 시사하였다.

Key words : cat, ginseng PD saponin, interleukin 8-like chemotactic activity, mononuclear cells, polymorphonuclear cells

Introduction

Interleukin (IL) 8, 8~10 kDa in molecular weight, is a potent neutrophil chemoattractant and also mitogenic and chemotactic for vascular smooth muscle cells[28]. It is secreted by numerous cells including monocytes, macrophage, epithelial cells, fibroblast, endothelial cells and keratinocytes[18-20,27]. The biological activities associated with IL 8 are neutrophil chemotaxis, phagocytosis of opsonized erythrocytes, inhibition of the proliferation of *Candida albicans*, the respiratory burst, synthesis of leukotriens, release of enzymes from granulocytes, expression of several members of the β_2-intergrin family, oxygen-independent mycobactericidal mechanisms of granulocytes[2-6,12,23].

On the other hand, the soluble products from activated monocytes and lymphocytes have been also considered to cause a cellular infiltration into inflamed sites such as arthritic joint and shown to directly

This work was supported by Grant-in-Aid from Korea Science and Engineering Fundation (KOSEF; 971-0605-042-1).

[1]Corresponding author.

induce chemotactic response for phagocytes[10,17]. It was shown that bacterial lipopolysaccharide (LPS), a mitogen, modulates multiple neutrophil function and that IL 1 released from peripheral blood mononuclear cells (MNC) is also associated with chemotaxis of phagocytes[25]. However, although the culture supernatant of LPS-stimulated monocytes enhanced a neutrophil chemotactic activity, highly purified or recombinanat IL 1 did not affect chemotactic activity of neutrophils[15]. Thus, it appears that chemotactic activity of neutrophil is mediated by other soluble products but not by IL 1 released from MNC exposed to mitogens. From these observations, it could be thought that soluble products, which is produced by MNC in response to ginseng saponins, especially ginseng PD saponin (GPD), will be associated with the IL 8-like chemotactic factor(s) for peripheral blood polymorphonuclear cells (PMNC). Therefore, feline IL 8-like chemotactic activity for PMNC in culture supernatant from MNC exposed to GPD was examined.

Materials and methods

Animals

Four healthy cats of average 1.2-year-old were housed at animal cage. All cats used were free from FeLV infection as examined by an enzyme-linked immunosorbent assay using a Leukassay F kit (Pitman Moore, NJ, USA).

Reagents

GPD was kindly donated by the Korea Ginseng and Tobacco Research Institute (Taejeon, Korea). Both human recombinant (r) IL 8 and anti-human IL 8 monoclonal antibody (mAb), IgG1, were commercially purchased (Sigma, MO, USA).

Feline MNC and PMNC

Buffy coat prepared from blood of healthy donors was diluted in phosphate-buffered saline (PBS) and then subjected to Lymphoprep (specific gravity, 1.077; Nycomed Pharma As, Oslo, Norway) density gradient centrifugation at 400×g for 40 min at room temperature. The cells in interface between PBS plus plasma and Lymphoprep were collected. This MNC was composed of both approximately 10% monocytes and 90% lymphocytes. The PMNC was purified by 1.5% dextran (molecular weight, 200,000; Wako, Osaka, Japan) sedimentation at room temperature for 60 min. The floating cells were collected and centrifuged at 400×g for 5 min. The remaining erythrocytes in isolation of MNC and PMNC were removed by 0.83% NH_4Cl solution and washed 3 times with PBS. The purity of neutrophils in final PMNC suspension was 96% when determined by cytospin smear and Giemsa stain. Cell viabilities determined by trypan blue dye exclusion always exceeded 98%. All cells were suspended in RPMI 1640 (Sigma, MO, USA) supplemented with 2 mM L-glutamine, 0.02 mg/ml of gentamicin, and 5% fetal bovine serum (FBS; Gibco, MD, USA).

Culture supernatant from MNC exposed to GPD

The MNC at a density of 2×10^6 cells/ml in a well of a 24-multiwell plate (Falcon 3047, Becton Dickinson Labware, NJ, USA) was incubated with concentration of 200 μg/ml of GPD for 24 hr at 37°C under 5% CO_2-humidified atmosphere. Culture supernatant was 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 for assay.

Chemotaxis assay

The PMNC chemotaxis was assayed by a modified Boyden chamber method[24]. The migrated distance of PMNC was measured by microscopy at 400× magnification. The chemotactic responsiveness of PMNC was evaluated as absolute distances (μm/45 min) in the directional migration of PMNC in response to chemoattrants.

Chemotactic response of feline PMNC by human rIL 8

Human rIL 8 was dissolved at 100 nM and diluted with PBS to 0.01, 0.1, 1 and 10 nM, respectively. Chemotactic activity of feline PMNC to the diluted

human rIL 8 was also tested.

Neutralization test with anti-human IL 8 mAb

Anti-human IL 8 mAb diluted with various concentrations was added to the culture supernatant of feline MNC exposed to GPD. The mixed culture supernatant was placed for 30 min at room temperature and the chemotactic activity to feline PMNC was also evaluated.

Physicochemical analyses

To test the temperature stability, culture supernatant of MNC exposed to GPD was heated at the temperature of 4, 20, 37, 60 and 100°C for 30 min. Culture supernatant was also dialysed against PBS at pH 3.0, pH 7.3 and pH 9.0 at room temperature for 4 hr and thereafter, culture supernatant was re-dialysed against PBS at pH 7.3 at room temperature for 4 hr. After each treatment, the chemotactic activity for feline PMNC in the treated culture supernatants was determined.

Data analysis

The Student's *t* test was used for statistical significance determinations. All data expressed mean±SEM.

Results

Chemotactic response of feline PMNC by human rIL 8

To examine the similarity of biological effect between chemotactic activity by culture supernatant from MNC exposed to GPD and that of human rIL 8, the chemotactic activity of feline PMNC to human rIL 8 was evaluated. As shown in Fig 1, human rIL 8 also enhanced the chemotactic activity of feline PMNC at concentrations of 0.1 to 10 nM ($p < 0.01$) in a dose-response manner. This activity of feline PMNC to human rIL 8 at concentration of 10 nM was equivalent to that of culture supernatant (25%) from MNC exposed to GPD.

Neutralization test with anti-human IL 8 mAb

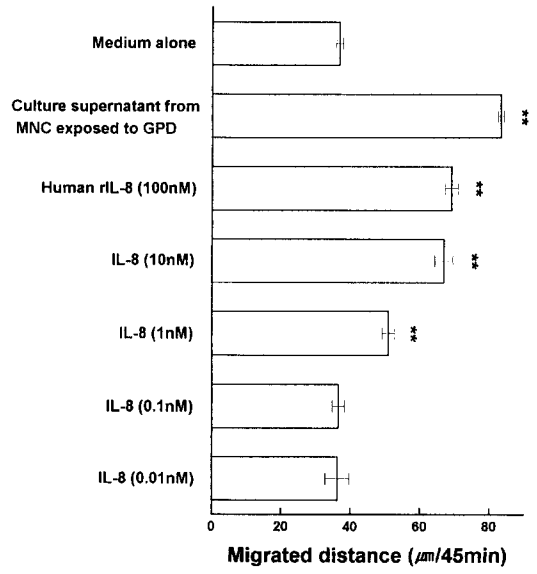

Fig 1. Chemotactic responses of feline PMNC to human rIL 8 at concentrations of 0.01 to 100 nM and culture supernatant (25%) from MNC exposed to GPD. The values represent mean±SEM (n=3). **p<0.01, compared to medium alone.

Neutralization effect of anti-IL 8 mAb in enhanced PMNC chemotaxis in response to culture supernatant (25%) from MNC exposed to GPD and human rIL 8 at a concentration of 10 nM was also examined. The chemotactic activity in culture supernatant was inhibited ($p<0.01$) in a dose-dependent manner by addition of anti-IL 8 mAb at concentations of 0.5 to 50 μg/ml when compared with that in culture supernatant (25%) from exposed to GPD (Fig 2). Similarly, the inhibitory effect of anti-IL 8 mAb was also observed in the chemotactic activity of feline PMNC to human rIL 8.

Physicochemical analyses

To investigate the physicochemical characteristics of culture supernatant from MNC exposed to GPD, the temperature stability for chemotactic activity and its resistance to acid and alkaline treatments were tested. The chemotactic activity by culture supernatant from MNC exposed to GPD was unaffected by temperatures between 4 and 100°C for 30 min (Fig 3) and it was also found to be stable at pH 3.0 and pH

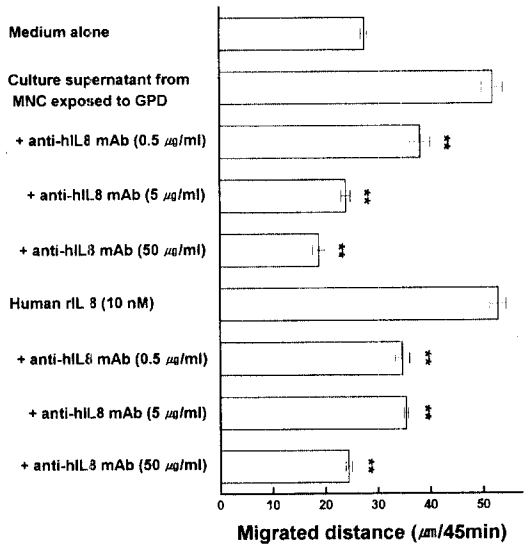

Fig 2. Effect of anti-human IL 8 mAb on feline PMNC by culture supernatant (25%) from MNC exposed to GPD and human rIL 8 (10 nM). The values represent mean± SEM (n=3). **p<0.01, compared to culture supernatant (25%) from MNC exposed to GPD and human rIL 8 (10 nM).

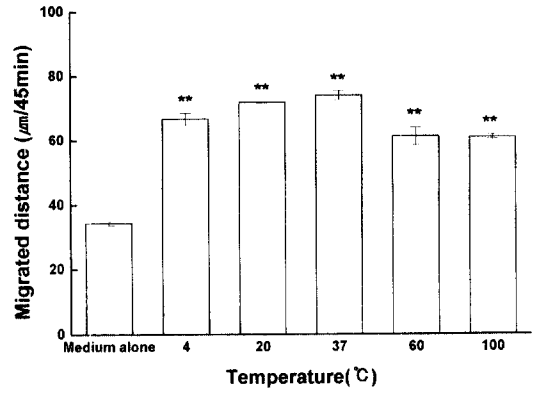

Fig 3. Effect of heat on chemotactic response of feline PMNC by culture supernatant (25%) from MNC exposed to GPD. The values represent mean±SEM (n=3). **p< 0.01, compared to medium alone.

9.0 buffer for 4 hr (Fig 4).

Discussion

The enhancing effect of chemotaxis by ginseng saponins in man was reported that the *in vivo* adminstration of the standardized ginseng extract G115

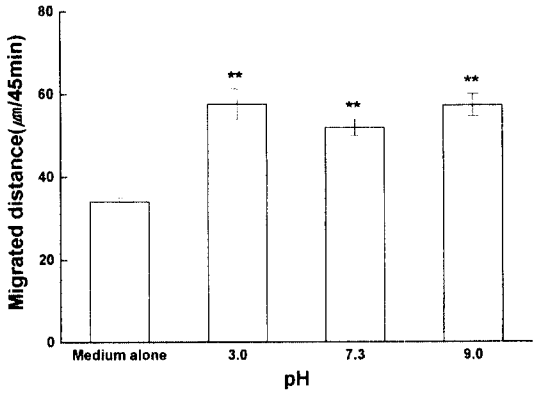

Fig 4. Effect of acid and alkaline treatment on chemotactic response of feline PMNC by culture supernatant (25%) from MNC exposed to GPD. The values represent mean± SEM (n=3). **p<0.01, compared to medium alone.

and alternative ginseng extract PKC 169/79 induced the neutrophils chemotaxis[14]. Our results demonstrated that GPD is able to release the chemoattractant(s) from MNC containing both monocytes and lymphocytes because culture supernatant from MNC treated with GPD enhanced the chemotactic activity for PMNC. Therefore, GPD is capable of stimulating non-specific immune response in cat.

Certain cytokines are capable of crossing the species barrier, e.g., human IL 2 shows cross-reactive effect on mouse cells and bovine peripheral blood MNC[1,26]. Human IL 6 actually promoted the differentiation of feline MNC, indicating that feline cells may react to human IL 8[7]. Thus, it was determined if human rIL 8 and anti-human IL 8 mAb exert any effect or action on the feline PMNC chemotaxis. In conclusion, feline PMNC chemotaxis was also induced by human rIL 8. In addition, anti-human IL 8 mAb at concentration of 10 nM inhibited completely the feline PMNC chemotaxis which was induced by culture supernatant of MNC exposed to GPD and human IL 8. The present results revealed that the physicochemical properties in culture supernatant of GPD-exposed MNC were stable in heat, acid and alkali. These facts were in agreement with those of human IL 8[8,13,16,22]. From these both biological effect and physicochemical properties, it was strongly suggested that feline IL 8-like chemotactic factor(s) exist in culture supernatant from MNC exposed to GPD.

PMNC chemotactic response to human rIL 8 was examined in various species[11,21,29]. The migration of human, monkey, hamster and dog neutrophils to human rIL 8 exhibited the increased chemotactic activities. Rabbit, rat and mouse neutrophils showed a low responsiveness to human rIL 8. In another study, however, anti-human IL 8 mAb and polyclonal antibodies to canine IL 8 did not inhibit the enhanced chemotactic response of PMNC by culture supernatant of MNC exposed to egg white derivatives (EWD)[9]. Thus, isolation and characterization of feline IL 8-like chemotactic factor(s) are needed to define the action mechanisms of PMNC chemotaxis by the culture supernatant of MNC exposed to GPD.

Conclusion

The IL 8-like chemotactic activity for feline PMNC by culture supernatant from MNC exposed to GPD was examined. The chemotactic activity was measured by a modified Boyden chamber assay. Culture supernatnat from feline MNC exposed to GPD enhanced the chemotactic activity for feline PMNC. The chemotactic activity of feline PMNC was also induced by human recombinant (r) IL 8. In addition, PMNC chemotactic activities promoted by both culture supernatant from MNC exposed to GPD and human rIL 8 were inhibited in a dose-dependent manner by addition of anti-human IL 8 monoclonal antibody. The physicochemical characteristics of chemotactic activity of PMNC were stable in heated (60 to 100°C), acidic (pH 3.0) and alkaline (pH 9.0) conditions. These findings for biological effect and physicochemical properties in culture supernatant from MNC exposed to GPD was quite similar to those of human IL 8. Therefore, these results strongly suggested that feline IL 8-like chemotactic factor(s) exist in culture supernatant from MNC exposed to GPD.

References

1. Atluru D, Xue W, Polam S, Atluru S, Blecha F, Minocha HC. *In vitro* interactions of cytokines and bovine viral diarrhea virus in phytohemagglutinin-stimulated bovine mononuclear cells. Vet Immunol Immunolpathol 1990; 25: 47-59.
2. Bazzoni F, Cassatella MA, Rossi F, Ceska M, Dewald B, Bagiolini M. Phagocytozing neutrophils produce and release high amounts of neutrophil-activating peptide NAP-1/IL-8. J Exp Med 1991; 173: 771-774.
3. Detmers PA, Lo SK, Olsen EE, Waltz A, Baggiloini M, Cohn ZA. Neutrophil-activating protein 1/interleukin 8 stimulates the binding activity of the leukocyte adhesion receptor cd11b/cd18 on human neutrophils. J Exp Med 1990; 171: 1155-1162.
4. 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.
5. 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.
6. Djeu JY, Matsushima K, Oppenheim JJ, Shiotsuki K, Blanchard DK. Functional activation of human neutrophil by recombinant monocyte-derived neutrophil chemotactic factor/IL-8. J Immunol 1990; 144: 2205-2210.
7. Goitsuka R, Ohashi T, Ono K, Yasukawa K, Koishibara Y, Fukui H, Ohsugi Y, Hasegawa A. IL-6 activity in feline infectious peritonitis. J Immunol 1990; 144: 2599-2603.
8. Hassfurther RL, Canning PC, Geib RW. Isolation and characterization of an iInterleukin-8-like peptide in the bovine species. Vet Immunol Immunopathol 1993; 42: 117-126.
9. Hirota Y, Mohamed A, Masumoto Y, Furusawa S, Yoshihara K, Masumoto Y, Suzuki K, Onodera T. Production and characterization of polyclonal anti-canine interleukin-8 antibodies. J Vet Med Sci 1996; 58: 219-224.
10. Kharazmi A, Nielsen H, Bendtzen K. Recombinant interleukin 1 alpha and beta prime human monocyte superoxide production but have no effect on chemotaxis and oxidative burst response of neutrophils. Immunobiology 1988; 177: 32-39.
11. Nancy EC, Frank DE, Roth JA. Effect of recombinant human cytokines on porcine neutrophil function. Vet Immunol Immunopathol 1992; 37: 39-47.
12. Nibbering PH, Pos O, Stevenhagen A, Furth RV. Interleukin-8 enhances nonoxidative intracellular killing of *Mycobacterium fortuitum* by human granulocytes. Infect Immun 1993; 61: 3111-3116.

13. Peveri P, Walz A, Dewald B, Baggiolini M. A novel neutrophil-activating factor produced by human mononuclear phagocytes. J Exp Med 1988; 167: 1547-1559.
14. Scaglione F, Ferrara F, Duanani S, Falchi M, Santoro G, Fraschini F. Immunomodulatory effects of two extracts of *Panax ginseng* C.A. Meyer. Drugs Exptl Clin Res 1990; XVI: 537-542.
15. Schmidt RJ. Purification and partial biochemical characterization of normal human interleukin 1, J Exp Med 1984; 160: 772.
16. Schroeder JM, Mrowietz U, Morita E, Christophers E. Purification and partial biochemical charaterization of a human monocyte-derived, neutrophil-activating peptide that lacks interleukin 1 activity. J Immunol 1987; 139: 3474-3483.
17. Seitz M, Dewald B, Gerber N, Baggiolini M. Enhanced production of neutrophil-activating peptide-1/interleukin-8 in rheumatoid arthritis. J Clin Invest 1991; 87: 463-469.
18. Standiford RM, Kunkel SL, Basha MA, Chensue SW, Lynch JP, Toews GB, Westwick J, Strieter RM. Interleukin-8 gene expression by a pulmonary epithelial cell line, a model for cytokine networks in the lung. J Clin Invest 1990; 86: 1945-1953.
19. Strieter RM, Chensue SW, Basha MA, Standiford TJ, Lynch JP, Baggiolini M, Kunkel SL. Human alveolar macrophage gene expression of interleukin-8 by tumor necrosis factor-alpha, lipopolysaccharide, and interleukin-1 beta. Am J Respr Cell Mol Biol 1990; 2: 321-326.
20. Strieter RM, Phan SH, Showell HJ, Remick DG, Lynch JP, Genord M, Rainford C, Eskandari M, Marks RM, Kunkel SL. Monokine-induced neutrophil chemotactic factor gene expression in human fibroblast. J Biol Chem 1989; 264: 10621-10626.
21. Sugawara T, Miyamoto M, Takayama S, Kato M. Separation of neutrophils from blood in human and laboratory animals and comparison of the chemotaxis. J Pharmacol Toxicol Meth 1995; 33: 91-100.
22. Uchiyama T, Ito A, Ikesue A, Nakagawa H, Mori Y. Chemotactic factor in the pregnant rabbit uterine cervix. Am J Obstet Gynecol 1992; 167: 1417-1422.
23. Ueno A, Murakami K, Yamanouchi K, Watanabe M, Kondo T. Thrombin stimulates production of interleukin-8 in human umbilical vein endothelial cells. Immunology 1996; 88: 76-81.
24. Watanabe K., Nakagawa H, Tsurufuji S. A new simple plastic chemotaxis device of the boyden chamber type utilizing an immunoassay plate. Jan J Pharmacol 1985; 39: 102-104.
25. Wuyts A, Proost P, Put W, Lenaerts JP, Paemen L, Damme VJ. Leukocyte recruitment by monocyte chemotactic protein (MCPs) secreted by human phagocytes. J Immun Method 1994; 174: 237-247.
26. Yang M.P.(1991) Studies on the growth factor for bovine leukemia virus-Infected lymphoblastoid B-cell lines. in PhD. thesis.
27. Yoshimura T, Masushima K, Oppenheim JJ, Leonard JP. Neutrophil chemotactic factor produced by lipopolysaccharide(LPS)-stimulated human blood mononuclear leukocytes: Partial characterization and separation from interleukin 1(IL-1). J Immunol 1987; 139: 788-793.
28. Yue TL, Wang XK, Sung CP, Olson B, McKenna PJ, Gu JL, Feuerstein GZ. Interleukin-8 a mitogen and chemoattractant for vascular smooth muscle cells. Circ Res 1994; 75: 1-7.
29. Zwahlen RD, Spreng D, Wyder-Walther M. *In vitro* and *in vivo* of human interleukin-8 in dogs. Vet Pathol 1994; 31: 61-66.