J. Exp. Biomed. Sci. 15 (2009) 93–96

Brief Communication

Chemotactic Effect of the House Dust Mite Allergen, *Dermatophagoides pteronyssinus* on Human Monocytic THP-1 Cells

Eun Ju Yang[1], Ji-Sook Lee[2], Chi-Young Yun[2] and In Sik Kim[1,†]

[1]*Department of Biomedical Laboratory Science, School of Medicine, Eulji University, Daejeon 301-832, Korea.*
[2]*Department of Biology, College of Natural Sciences, Daejeon University, Daejeon 300-716, Korea*

House dust mites (HDMs) play an important role in the occurrence of allergic diseases such as asthma and atopic dermatitis. *Dermatophagoides pteronyssinus* (*Der p*) is one of the most prevalent HDMs. It mediates the activation of T cells and monocytes, and induces the elevation of immunoglobulin E levels in allergic diseases. However, the effects of *Der p* on human monocytes have not been fully understood. In the present study, we investigated whether or not *Der p* has a great effect on the chemotactic activity of the human monocytic cell line, THP-1 cells, as induced by CC chemokines. We also show that the *Der p* extract (DpE) increased the chemotactic activity of THP-1 cells in response to MCP-1, RANTES, MIP-1α, and TARC, but had no effect on the expressions of CC chemokine receptors (CCRs) binding to CC chemokines in THP-1 cells. Protease inhibitors, such as aprotinin and E64, blocked the increased chemotaxis, while cytoplasmic Ca^{2+} influx mediated by these chemokines was inhibited by DpE. These results indicate that DpE increases the chemotactic activity of THP-1 cells in response to CC chemokines by regulating the cells' protease-dependent mechanism. This finding may be useful in identifying the pathogenesis of allergic diseases induced by *Der p*.

Key Words: House dust mite, Monocytes, Chemotactic activity, CC chemokine

House dust mites (HDMs) are the main allergens in a variety of allergic diseases, including asthma, atopic dermatitis, and rhinitis (Arlian and Platts-Mills, 2001). These are ubiquitous, with *Dermatophagoides pteronyssinus* (*Der p*) (Roche et al., 1997) being one of the most prevalent. Studies show that most asthma and atopic dermititis patients have positive reactions to the *Der p* skin prick tests (Ring and Darsow, 2001). In these patients, *Der p* induces the high level of immunoglobulin E in the serum and mediates the proliferation and activation of T cell (Friedmann, 1999). Human monocytic THP-1 cells release high levels of MCP-1, IL-6, and IL-8 after the stimulation of *Der p* (Lee et al., 2008). In chronic asthma, monocytes and macrophages induce various inflammatory mediators and reactive oxygen that mediate tissue damage and contribute to the pathogenesis of the asthma (Holgate, 2008). Similarly, monocytes in the atopic dermatitis migrate into the skin due to the monocyte chemotactic protein-1 (MCP-1) and are differentiated into the dendritic epidermal cells that produce various inflammatory cytokines (Bieber, 2008). It is clear that the migration and activation of monocytes are important immune responses in allergic diseases. Among various chemoattractants, members of the CC(β) chemokine family have been reported to increase the chemotactic activity of the monocyte lineage, while CXC(α) chemokines induce neutrophil migration (Taub and Oppenheim, 1994). These CC chemokines include MCP-1, macrophage inflammatory protein 1α (MIP-1α), regulated on activation normal T expressed and secreted (RANTES), and thymus- and activation-regulated chemokine (TARC) and they are involved in allergic disease (Murphy et al., 2000). Although monocyte migration is an important process in the allergic sites, the precise role of *Der p* in the chemotaxis of human monocytes has not been completely elucidated.

In the present study, THP-1 cells were obtained from the American Type Culture Collection (Manassas, VA) and were maintained in RPMI 1640 supplemented with 10%

*Received: February 3, 2009
Accepted after revision: March 15, 2009
†Corresponding author: In Sik Kim, Department of Biomedical Laboratory Science, School of Medicine, Eulji University, 143-5 Yeuongdu-dong, Jung-gu, Daejeon 301-746, Korea.
Tel: +82-42-259-1753, Fax: +82-42-259-1759
e-mail: orientree@eulji.ac.kr

heat-inactivated fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 μg/ml). The cells were used as in vitro model of human primary monocytes in this study. The *Der p* extract (DpE) was supplied by Dr. Tai-Soon Yong (Yonsei University College of Medicine, Seoul, Korea). Endotoxin in DpE was removed by Endo Trap Red (Lonza, MD), according to the manufacturer's instructions. Eodotoxin level in DpE was very low (0.005 EU/mg of DpE) as compared with that reported by other studies (<0.96 EU/mg of DpE) (Liu et al., 2005). To determine the effect of DpE on the migration of THP-1 cells induced by CC chemokines such as MCP-1, RANTES, MIP-1α and TARC, we performed a chemotaxis assay using a 48-well microchamber (Neuroprobe, Gaithersburg, MD) and a polyvinylpyrrolidone-free filter (Neuroprobe) with a pore-size of 5 μm. The cells of four randomly selected fields per well were counted, and the chemotactic index (CI) was calculated from the number of cells that migrated to the control. In order to detect the mRNA levels of CC chemokine receptors (CCRs) in THP-1 cells, the total RNA was extracted from THP-1 cells using a Trizol reagent, after which reverse-transcriptase polymerase chain reaction (RT-PCR) was carried out. We also performed a flow cytometry for the detection of CCR surface expression, which was then analyzed by CellQuest software on a FACSCalibur (Becton Dickinson, Franklin Lakes, NJ). There were 10,000 events collected for each experiment. To monitor the alternations in intracellular free calcium (Ca^{2+}) level following chemokine stimulation, THP-1 cells were incubated with the Ca^{2+} indicator fluo-3 acetoxylmethyl (fluo-3 AM) ester and were run at a concentration of >500 cells/second on flow cytometry. Fluorescence increase was evaluated every second for 120 seconds. In this study, data were expressed as the means ± S.E.M, and statistical

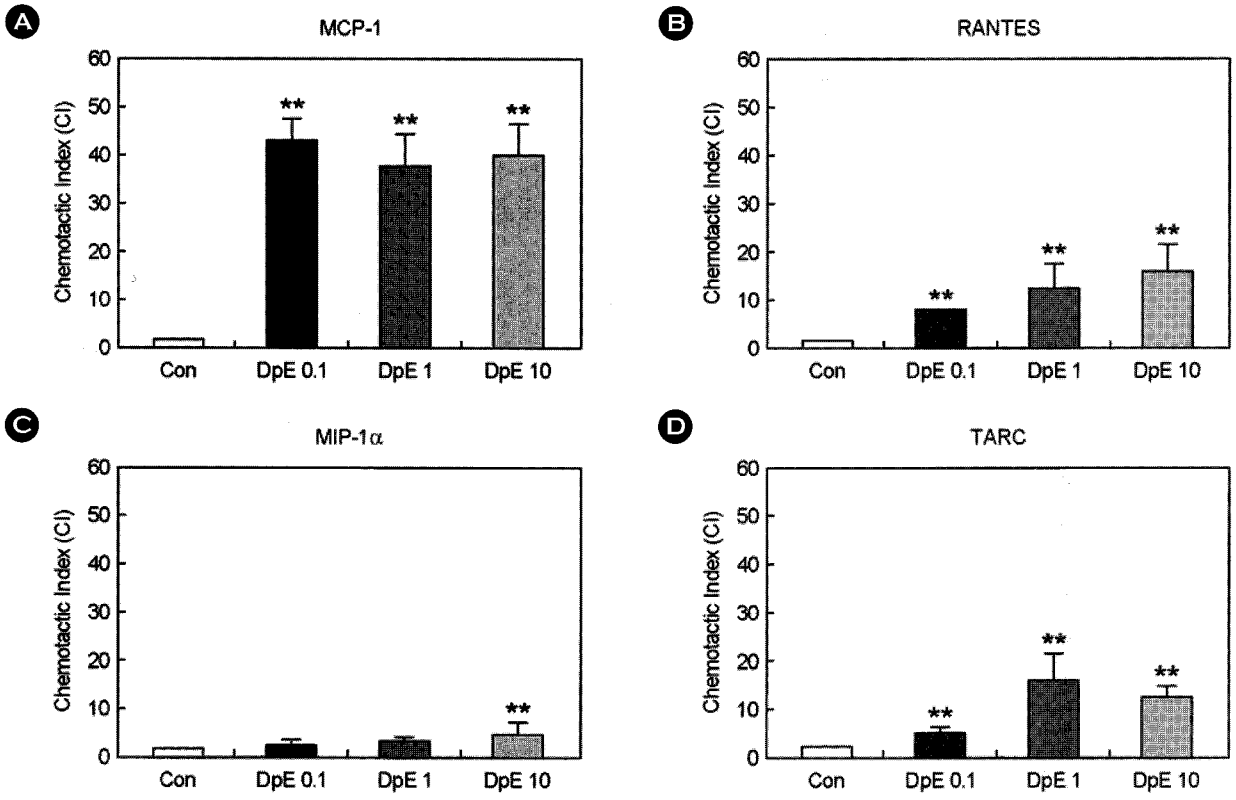

Fig. 1. The effects of DpE on THP-1 cell migration in response to MCP-1, RANTES, MIP-1α, and TARC. THP-1 cells were starved for 24 h at 37℃ in RPMI 1640 containing 0.5% FBS. The cells were treated with DpE in a dose-dependent manner for 24 h and were incubated with MCP-1 (100 ng/ml), RANTES (100 ng/ml), MIP-1α (10 ng/ml), and TARC (1000 ng/ml) in a 48-well microchamber for 5 h. After removing the non-migrated cells that adhered on the upper surface of the polycarbonate filter, this filter was then stained with Diff-Quick. The chemotactic index (CI) was calculatd from the number of cells that migrated to the control. A single experiment includes six replicate measurements, and data are expressed as the mean CI ± S.E.M. Here, $^{**}P<0.01$ was considered a significant difference between the untreated group and the DpE-treated group.

differences were analyzed using the paired t-test. The SPSS statistical software package (Version 10.0, Chicago, IL) was used for statistical analysis, in which a significant *P* value was defined as less than 0.01.

We first determined the chemotactic activity of THP-1 cells induced by MCP-1, RANTES, MIP-1α, and TARC. The chemotactic activities of THP-1 cells induced by CC chemokines have been reported in previous studies (Cross et al., 1997; Gouwy et al., 2008). As shown in Fig. 1, DpE increased the migration of THP-1 cells in response to MCP-1, RANTES, MIP-1α, and TARC and that the increased activity was dependent on the concentration of DpE. In particular, MCP-1 strongly induced the chemotactic activity of THP-1 cells following DpE stimulation. THP-1 cells express surface CCR2 protein among CCR subtypes, resulting in interaction of MCP-1 with CCR2 (Gouwy et al., 2008). To investigate the precise mechanism behind the chemotactic effect of DpE, we examined the mRNA and surface protein expressions of CCR1 through CCR5 expressions after treatment with DpE in a dose-dependent manner. Although the mRNA levels of CCR1-5 were slightly reduced by DpE, it did not change the surface expression levels of these receptors (Fig. 2A and B). These results indicate that the elevated chemotactic activity

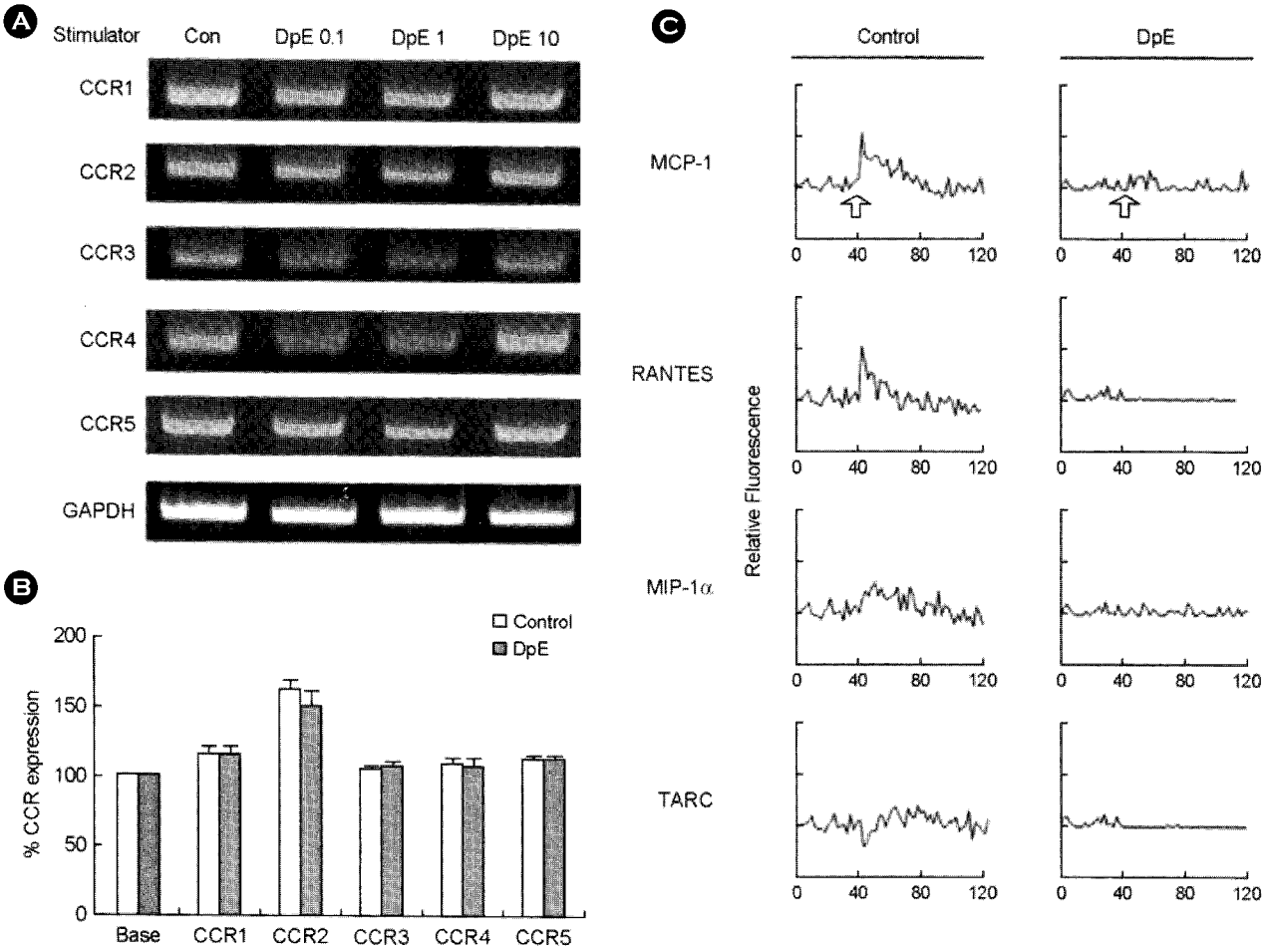

Fig. 2. The effects of DpE on CCR expression and Ca^{2+} influx in THP-1 cells. **A,** Total mRNA was extracted from THP-1 cells after DpE treatment for 24 h in a dose-dependent manner, after which RNA levels of CCR subtypes were analyzed by RT-PCR. We used GAPDH as an internal control, and expressed the data as representative of six individual experiments. **B,** The surface protein expressions of CCR subtypes in THP-1 cells treated with DpE (10 μg/ml) for 24 h were analyzed by flow cytometry using anti-CCR subtypes antibodies. Baseline was analyzed by incubating only the normal mouse IgG. The relative surface protein expression of CCR subtypes (taking the fluorescence level of baseline as 100%) shows the means ± S.E.M. **C,** The alternation of intracellular Ca^{2+} concentration in THP-1 cells was analyzed by staining with the Ca^{2+} indicator fluo-3 AM. The cells were incubated with or without DpE (10 μg/ml) for 24 h before treatment with CC chemokine. The fluorescence levels were measured every second for 120 seconds.

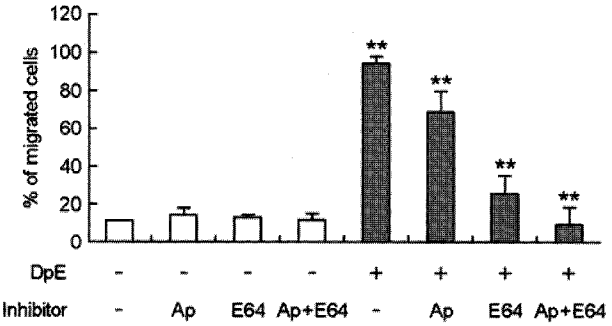

Fig. 3. Chemotactic activity increased by DpE is associated with the protease-mediated mechanism. Serum starved THP-1 cells were pre-incubated in the absence or presence of 50 μg/ml aprotinin (Ap), 50 μg/ml E64, or 50 μg/ml aprotinin plus 50 μg/ml E64 for 30 min. After treatment with 10 μg/ml DpE for 24 h, the cell migration assay was performed. Data are expressed as the mean CI ± S.E.M. Here, $^{**}P<0.01$ was considered a significant difference between the untreated group and the DpE-treated group or between the DpE-treated group and the inhibitor-treated group.

induced by DpE is not associated with alternations of CCR expression and may instead be influenced by other responses. In the calcium influx experiments, MCP-1 and RANTES showed immediate and potent response without DpE stimulation. The calcium influx was slightly induced by MIP-1α but not by TARC (Fig. 2C). Meanwhile, DpE stimulation slowed down the increase of intracellular calcium concentration due to the presence of CC chemokines in THP-1 cells. Given that many chemokines induce an elevation of the calcium cytoplasmic level after binding to their receptors (Ling et al., 1999), these data are consistent with the results that DpE has no effect on CCR expression. As shown in Fig. 3, the cell migration increased by DpE was significantly blocked by aprotinin, a serine protease inhibitor, and E64, a cysteine protease inhibitor ($P<0.01$). Although it has been shown that protease-dependent compounds in DpE may be involved in DpE-induced chemotaxis, nevertheless, there is a need to further identify the associated protease in a future study.

In conclusion, we demonstrate that *Der p* may change allergic response by modulating the chemotactic activitiy of human monocytes. This study may contribute to further understanding the developmental processes in *Der p*-sensitive allergic diseases.

Acknowledgements

This work was supported by the RIC program of MKE (Ministry of Knowledge Economy) in Daejeon University.

REFERENCES

Arlian LG, Platts-Mills TAE. The biology of dust mites and the remediation of mite allergens in allergic disease. J Allergy Clin Immunol. 2001. 107: S406-S413.

Bieber T. Atopic dermatitis. N Engl J Med. 2008. 358: 1483-1494.

Cross AK, Richardson V, Ali SA, Palmer I, Taub DD, Rees RC. Migration responses of human monocytic cell lines to α- and β-chemokines. Cytokine 1997. 9: 521-528.

Friedmann PS. The role of dust mite antigen sensitization and atopic dermatitis. Clin Exp Allergy 1999. 29: 869-972.

Gouwy M, Struyf S, Noppen S, Schutyser E, Springael JY, Parmentier M, Proost P, Damme JV. Synergy between coproduced CC and CXC chemokines in monocyte chemotaxis through receptor-mediated events. Mol Pharmacol. 2008. 74: 485-495.

Holgate ST. Pathogenesis of asthma. Clin Exp Allergy 2008. 38: 872-897.

Lee JS, Kim IS, Ryu JS, Yun CY. House dust mite, Dermatophagoides pteronissimus increases expression of MCP-1, IL-6 and IL-8 in human monocytic THP-1 cells. Cytokine 2008. 42: 365-371.

Ling K, Wang P, Zhao J, Wu YL, Cheng ZJ, Wu GX, Hu W, Ma L, Pei G. Five-transmembrane domains appear sufficient for a G protein-coupled receptor: Functional five-transmembrane domain chemokine recpetors. Proc Natl Acad Sci USA. 1999. 96: 7922-7927.

Liu CF, Chen YL, Chang WT, Shieh CC, Yu CK, Reid KB, Wang JY. Mite allergen induces nitric oxide production in alveolar macrophage cell lines via CD14/toll-like receptor 4, and is inhibited by surfactant protein D. Clin Exp Allergy 2005. 35: 1615-1624.

Murphy PM, Baggiolini M, Charo IF, Hébert CA, Horuk R, Matsushima K, Miller LH, Oppenheim JJ, Power CA. International union of pharmacology. XXII. Nomenclature for chemokine receptors. Pharmacol Rev. 2000. 52: 145-179.

Ring J, Darsow U. Role of aeroallergens in atopic eczema: proof of concept with the atopy patch test. J Am Acad Dermatol. 2001. 45: S49-S52.

Roche N, Chinet TC, Huchon GJ. Allergic and nonallergic interactions between house dust mite allergens and airway mucosa. Eur Respir J. 1997. 10: 719-726.

Taub DD, Oppenheim JJ. Chemokines, inflammation and the immune system. Ther Immunol. 1994. 1: 229-246.