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Brief Communication

The House Dust Mite Allergen, *Dermatophagoides pteronyssinus* Suppresses the Chemotactic Activity of Human Monocytes

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House dust mite (HDM) is important in the pathogenesis of allergic diseases including asthma and atopic dermatitis. *Dermatophagoides pteronissinus* (Dp) is one of major HDM allergens. In this study, we investigated that Dp extract (DpE) affects on the chemotactic activity of monocytes isolated from the peripheral blood. DpE inhibited the migration of human monocytes in response to CC chemokines such as MIP-1α, RANTES, HCC-4, MCP-1, and TARC. DpE did not alter the expression of CC chemokine receptors (CCRs) such as CCR1, CCR2, CCR3, CCR4, and CCR5. These results indicate that DpE blocks the chemotaxis of human monocytes and its mechanism is not involved in alteration of CCR expression. Better understanding of the effect of DpE on monocytes will enable elucidation of the role of Dp in the development of allergic diseases.

Key Words: House dust mite, Monocytes, Chemotaxis, CC chemokine

House dust mites (HDMs) are the essential allergens in various allergic diseases including asthma, and atopic dermatitis (Gaffin and Phipatanakul, 2009). HDM induces the production of HDM-specific IgE. Allergic patients represent an increased level of HDM IgE and total IgE in serum (Willart and Lambrecht, 2009). *Dermatophagoides pteronyssinus* (Dp) is one of the most prevalent HDMs (Roche et al., 1997). Dp induces the high level of immunoglobulin E in serum and mediates the proliferation and activation of T cell (Friedmann, 1999). We have recently reported that human monocytic THP-1 cells release high levels of MCP-1, IL-6, and IL-8 after the stimulation of Dp (Lee et al., 2008).

Chemokines are grouped into the four different families CXC, CC, C, and CX3C by the location of two cysteines (Baggiolini et al., 1997). CC chemokines have been reported to increase the chemotactic activity and survival of the monocytes and lymphocytes. These CC chemokines include macrophage inflammatory protein 1α (MIP-1α), regulated on activation normal T expressed and secreted (RANTES), human CC chemokine-4 (HCC-4), monocyte chemoattractant protein-1 (MCP-1), and thymus- and activation-regulated chemokine (TARC) and they are associated with allergy (Murphy et al., 2000). Because Dp extract (DpE) induces the migration the human monocytic cell line, THP-1 cells. we examined whether DpE stimulates the migration of human monocytes in the present study (Yang et al., 2009).

Monocytes were obtained from the heparinized whole blood of healthy donors. After peripheral blood mononuclear cells were separated by centrifugation over Ficoll-Hypaque density gradient, monocytes were purified using monocyte negative isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). Viability as assessed by trypan blue stain was

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>99% for isolated cells. The cells were maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 μg/ml). DpE was supplied by the Korea National Arthropods of Medical Importance Resource Bank (Seoul, Korea). To determine the effect of DpE on the monocyte migration in response to CC chemokines such as MIP-1α, RANTES, HCC-4, MCP-1, and TARC, we performed a chemotaxis assay using a 48-well microchamber (Neuroprobe, Gaithersburg, MD) and a polyvinylpyrrolidone-

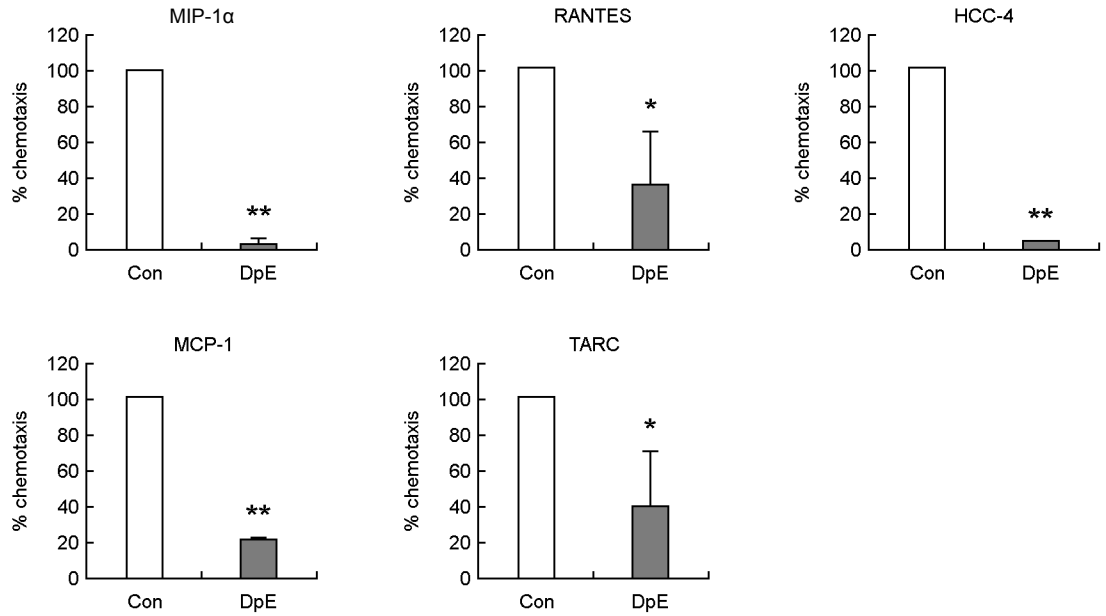

Fig. 1. The effects of DpE on monocyte migration in response to MIP-1α, RANTES, HCC-4, MCP-1, and TARC. Monocytes were isolated from the peripheral blood and were treated with DpE (10 μg/ml) for 24 h. The cells were incubated with MIP-1α (10 ng/ml), RANTES (100 ng/ml), HCC-4 (1,000 ng/ml), MCP-1 (100 ng/ml), and TARC (1,000 ng/ml) in a 48-well microchamber for 3 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. Data are expressed as the means ± S.E.M. and are presented in relation to the control group, which was set at 100%. $^{*}P<0.05$ and $^{**}P<0.01$ was considered a significant difference between the control group and the DpE-treated group.

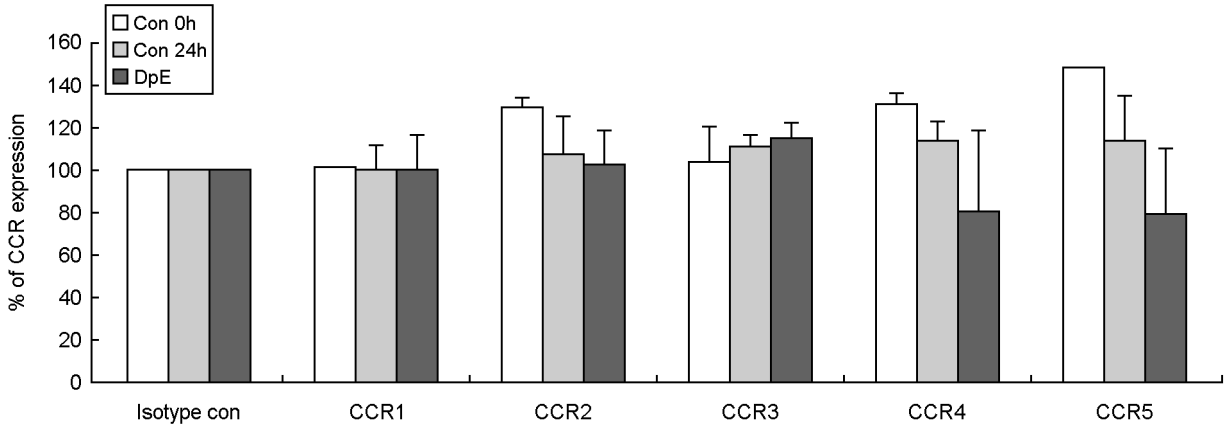

Fig. 2. The effects of DpE on CCR expression in monocytes. Isolated monocytes were treated with DpE (10 μg/ml) for 24 h. The protein expressions of CCR subtypes were analyzed by flow cytometry using anti-CCR subtypes antibodies. Baseline was analyzed by incubating only the normal mouse IgG. The relative surface protein expression of CCR subtypes (taking the fluorescence level of baseline as 100%) shows the means ± S.E.M.

treated membrane (Neuroprobe) with a pore-size of 5 μm. The cells of four randomly selected fields per well were counted. We performed a flow cytometry for the detection of CCR expression, and the cells was analyzed by CellQuest software on a FACSCalibur (Becton Dickinson, Franklin Lakes, NJ). There were 10,000 events collected for each experiment. In this study, data were expressed as the means ± S.E.M, and statistical 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 0.05.

We first investigated the monocyte migration induced by MIP-1α, RANTES, HCC-4, MCP-1, and TARC. The chemotactic activities of monocytes in response to CC chemokines have been reported in a previous study (Gouwy et al., 2008). Unexpectedly, DpE suppressed the monocyte migration induced by MIP-1α, RANTES, HCC-4, MCP-1, and TARC (Fig. 1). These results led us to examine whether DpE alters the expression of CCR1 through CCR5 binding to MIP-1α, RANTES, HCC-4, MCP-1 and TARC. DpE have no effect on the surface expression of CCRs (Fig. 2). In a previous study, we found that MIP-1α, RANTES, MCP-1, and TARC powerfully induce the chemotactic activity of THP-1 cells following DpE stimulation (Yang et al., 2009). Although the protein levels of CCR1-5 were not altered by DpE stimulation in THP-1 cells and monocytes, the chemotactic effect of DpE in monocytes is different from that in THP-1 cells. Because the difference is very important in physiological functions of the immune cells, we found limitation of the usage of the cell line as an in a *vitro* model in biological our research. DpE may induce inflammatory responses including cytokine release and activation of monocytes (Gaffin and Phipatanakul, 2009). Future investigation will need to examine the inflammatory effect of DpE in monocytes.

In conclusion, we demonstrate that DpE inhibits monocyte migration in response to CC chemokines and has no effect the expression of CCR1 through CCR5. This study may contribute to further understanding the association of Dp and monocytes in allergic diseases.

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