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Brief Communication

# Effect of House Dust Mite and CCL2 on S100A8 and S100A9 Expression in Human Monocytes

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The S100A8 and S100A9 proteins play important roles in inflammatory diseases. The house dust mite acts as a major allergen that induces allergic diseases. We investigated the effect of the house dust mite on S100A8 and S100A9 protein expression in monocytes. We also examined the effect of CCL2, a powerful monocyte chemoattractant, on the expression of both proteins. Extract of *Dermatophagoides pteronissinus* (DP), recombinant Der p 1 and Der p 2, or CCL2 had no effect on S100A8 and S100A9 expression in human monocytic THP-1 cells. Monocytes were isolated from healthy donors and treated with DP, Der p 1, and Der p 2. S100A8 expression in monocytes increased after a 24 h stimulation with DP, Der p 1, and Der p 2, and CCL2 also increased S100A8 production. However, S100A9 expression in monocytes was not altered by DP, Der p 1, Der p 2, or CCL2. These results indicate that house dust mite and CCL2 may trigger an inflammatory response by altering S100A8 expression.

**Key Words:** S100A8, S100A9, House dust mite, CCL2, Monocytes

The S100A8 and S100A9 proteins belong to the S100 protein family and the heterodimer formed by both proteins is called calprotectin (Vogl et al., 2012). They are highly expressed in the cytosol of neutrophils and monocytes. They are induced by several stimuli such as glucocorticoids and lipopolysaccharide. Double-stranded RNA induces S100A8 and S100A9 expression, and the increased protein expression is dependent on interleukin (IL)-10 in human monocytes and macrophages (Endoh et al., 2009). S100A8 and S100A9 are an intense focus of biomedical research because they are involved in cancer and allergic and autoimmune diseases (Goyette and Geczy, 2011; Srikrishna, 2012).

House dust mites induce allergic inflammation and aggravate diseases (Gould and Sutton; 2008). *Dermatophagoides pteronissinus* (DP) is an important species of house dust mite and includes a variety of proteins, mainly classified as group 1 and group 2. Der p 1 and Der p 2 are included in groups 1 and 2, respectively (Cui, 2013). Der p 1 acts as a cysteine protease and cleaves unknown target proteins. The cleaved proteins stimulate basophils following IL-4 production. IL-4 stimulates proliferation and activation of T helper type 2 (Th2) cell. Der p 2 interacts with TLR4, a S100A8 and S100A9 receptor, and induces both antigen presentation and cytokine secretion by dendritic cells (Thomas et al., 2010). CCL2 is one of the CC chemokines produced by endothelial cells, smooth muscle cells, and monocytes (Deshmane et al., 2009). CCL2 induces strong migration and activates monocytes expressing CCR2. We reported previously that the inflammatory response due to house dust mites is associated with increased CCL2 secretion (Lee et al., 2008).

Because our previous report showed that house dust mites are involved in regulating monocytes (Lee et al., 2012), and that S100A8 and S100A9 are associated with the inflammatory response, we hypothesized that house dust mites increase S100A8 and S100A9 expression in

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monocytes.

THP-1 cells used as an *in vitro* model of primary monocytes were obtained from the American Type Culture Collection (Manassas, VA, USA). They were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). Human monocytes were isolated from heparinized peripheral blood of healthy volunteers using Ficoll-Hypaque gradient centrifugation. The mononuclear layer was collected, and monocytes were negatively separated from lymphocytes using a magnetic cell sorting kit and lymphocyte-isolating cocktail microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). This study was approved by the Institutional Review Board of Eulji University.

THP-1 cells and monocytes were seeded at $3 \times 10^5$ cells/well and were cultured in RPMI 1640. After treatment with DP (10 μg/ml), Der p 1 (10 μg/ml), Der p 2 (10 μg/ml), and CCL2 (100 ng/ml) for 24 hrs, the cells were harvested and then resuspended in 100 μl of 4% paraformaldehyde solution. After removing the fixing solution, the cells were incubated with phosphate buffered saline (PBS) solution containing 0.01% Triton X-100. After washing three times with PBS solution, the cells were incubated with PBS buffer containing anti-S100A8 or anti-S100A9 (Abnova, Taipei, Taiwan). Baseline fluorescence values were obtained by incubating with normal mouse IgG instead of specific primary antibodies. After three washes, the cells were incubated at 4℃ for 30 min with FITC-conjugated goat anti-mouse IgG. Finally, the cells were washed and analyzed using a FACSort cytofluorimeter (BD Biosciences, San

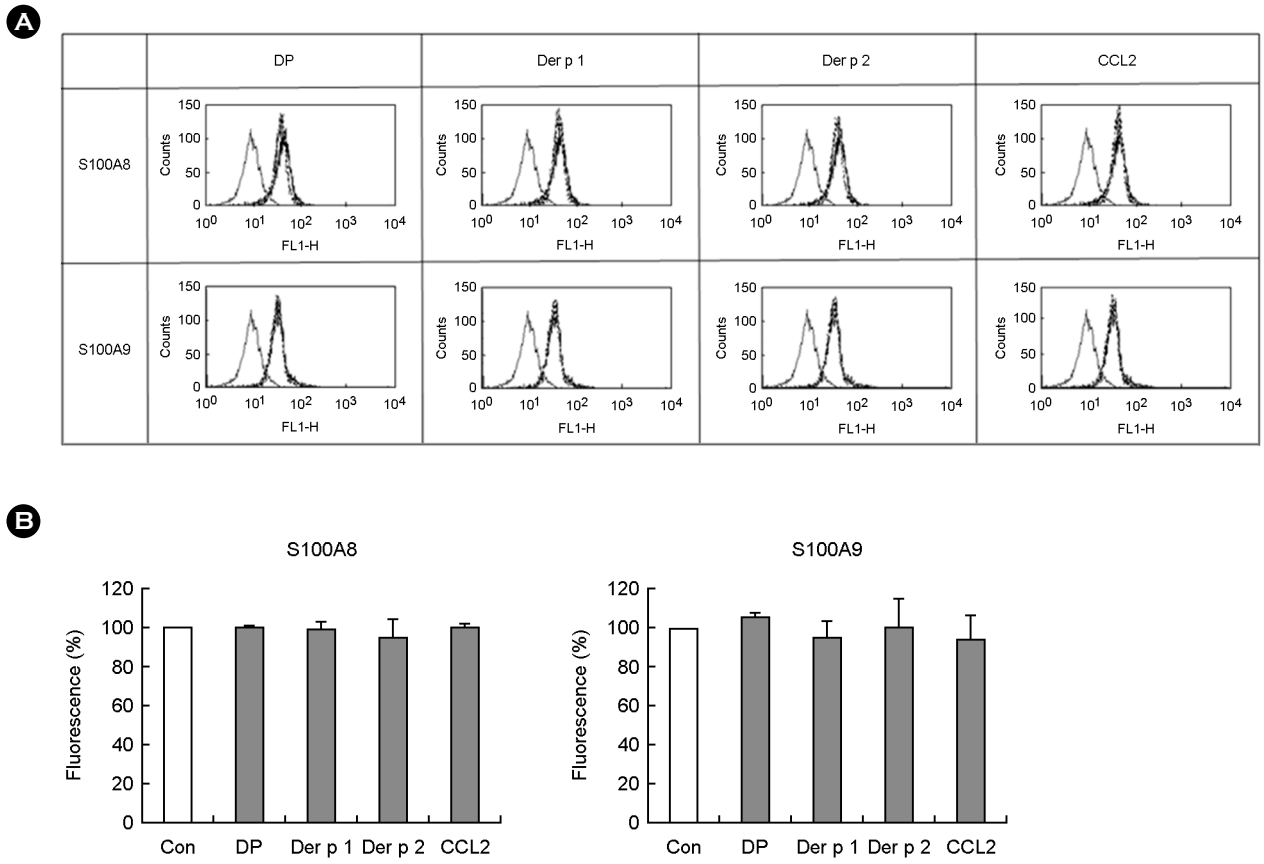


**Fig. 1. Efect of DP, Der p 1 and Der p 2, and CCL2 on S100A8 and S100A9 expression in THP-1 cells.** (A), THP-1 cells were treated without (thick line) and with 10 μg/ml Der p 1, 10 μg/ml Der p 2, 10 μg/ml DP, and 100 ng/ml CCL2 (dotted line). The cells were harvested and analyzed using a fluorescence-activated cell sorter with anti-S100A8 or anti-S100A9 antibodies. Baseline fluorescence values were obtained by incubation with normal mouse IgG (thin line). Reported data are representative of four individual experiments. (B), Data are presented in relation to the negative control (Con), which was set at 100% and are reported as the means ± SD of four separate experiments.

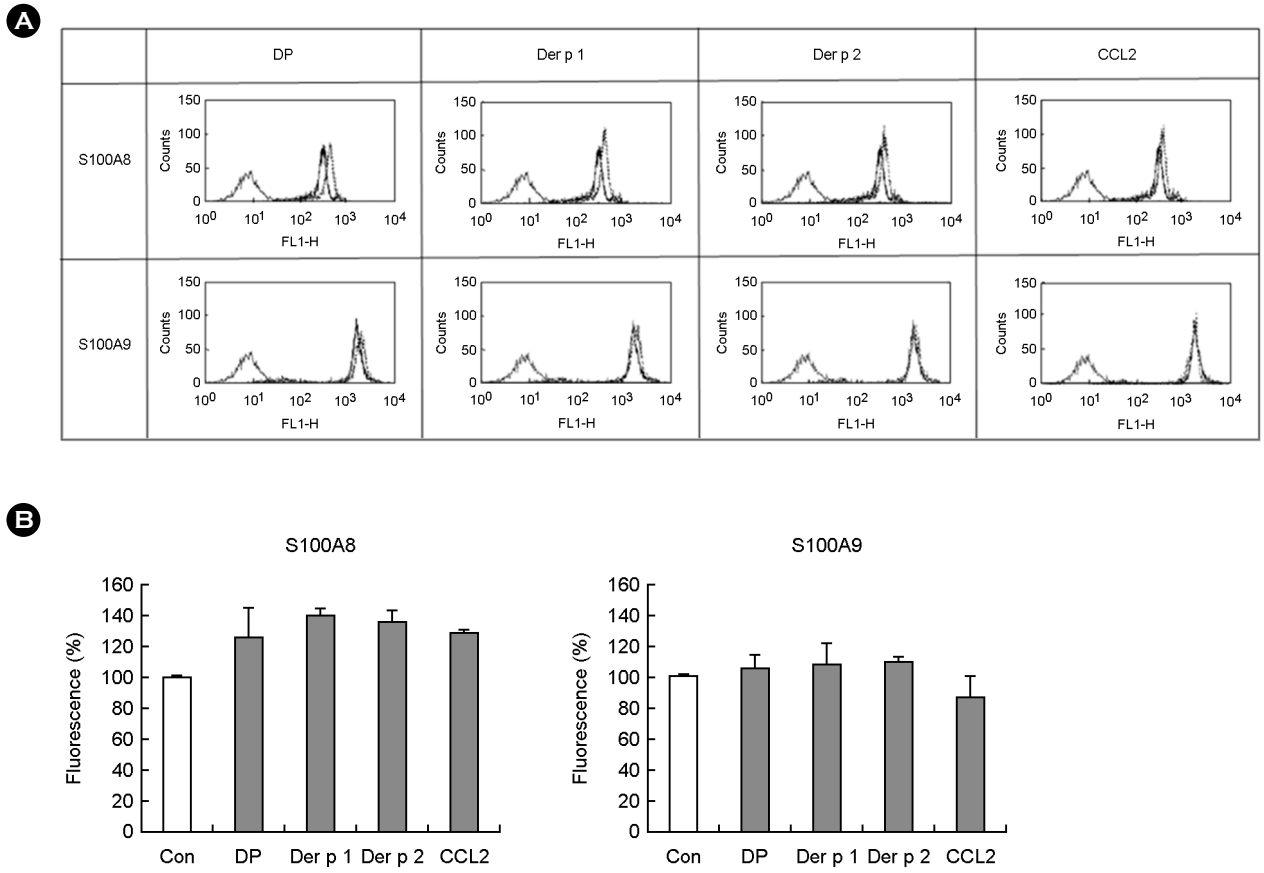


**Fig. 2. Efect of DP, Der p 1 and Der p 2 and CCL2 on S100A8 and S100A9 expression in monocytes.** (A), Isolated monocytes were treated without (thick line) and with 10 μg/ml Der p 1, 10 μg/ml Der p 2, 10 μg/ml DP, and 100 ng/ml CCL2 (dotted line). The cells were harvested and analyzed using a fluorescence-activated cell sorter with anti-S100A8 or anti-S100A9 antibodies. Baseline fluorescence values were obtained by incubation with normal mouse IgG (thin line). Reported data are representative of four individual experiments. (B), Data are presented in relation to the negative control (Con), which was set at 100% and are reported as the means ± SD of two separate experiments.

Diego, CA, USA). Ten thousand events were collected for each analysis. The DP extract was obtained from the Korea National Arthropods of Medical Importance Resource Bank (Yonsei University, Seoul, Korea). Der p 1 and Der p 2 were purchased from INDOOR Biotechnologies (Charlottesville, VA, USA). CCL2 was purchased from R&D Systems (Minneapolis, MN, USA).

We first examined the effect of house dust mites on S100A8 and S100A9 expression in THP-1 cells. As shown in Fig. 1, DP, Der p 1, and Der p 2 had no effect on S100A8 and S100A9 expression in THP-1 cells. We examined the alteration in S100A8 and S100A9 in human monocytes. S100A8 expression by isolated monocytes increased 27~40% after treatment with DP, Der p 1, and Der p 2. However, S100A9 expression was not altered by treatment with the stimulators (Fig. 2). These results indicate that house dust mites regulate S100A8 expression in monocytes. The house dust mite is strongly associated with asthma and atopic dermatitis (Gavino et al., 2008; Gaffin and Phipatanakul, 2009), and S100A8 and S100A9 are involved in asthma and atopic dermatitis (Roth et al., 1992; Halayko and Ghavami, 2009). As inferred from these reports and our results, the house dust mite may induce inflammatory diseases including asthma and atopic dermatitis by elevating S100A8 levels. Der p 1 and Der p 2 play essential roles altering S100A8 expression. Although the effect of CCL2 on S100A8 and S100A9 expression has not been reported, we used CCL2 as a comparative control in this study because CCL2 is a powerful factor in the monocyte inflammatory response. CCL2 altered S100A8 expression in monocytes. Increases

in the S100A8 protein due to CCL2 may be part of the inflammatory process induced by CCL2 as positive feedback (Melgarejoet al., 2009). In addition, the different actions of DP, Der p 1, Der p 2, and CCL2 between THP-1 cells and monocytes may indicate a limitation of the monocytic cell line and the importance of primary monocytes. The reason why S100A8 and S100A9 expression was differentially regulated by DP, Der p 1, Der p 2, and CCL2 is unknown in this study. Further study on the regulatory mechanism of S100A8 and S100A9 expression is required in the near future.

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