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Brief Communication

The House dust Mite Allergen, *Dermatophagoides pteronyssinus* Regulates the Constitutive Apoptosis and Cytokine Secretion of Human Eosinophils

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Asthma is an allergic inflammation and house dust mite (HDM) is a major allergen to induce asthma pathogenesis. Regulation of eosinophil apoptosis is an essential immune process and its dysregulation is implicated in asthma. In the present study, we examined the effects of HDM on spontaneous apoptosis of asthmatic eosinophils and on cytokine secretion in eosinophils of normal subjects including non-atopic and atopic normal. Extract of *Dermatophagoides pteronissinus* (DP) inhibited eosinophil apoptosis in a time-dependent manner. DP increased the secretion of G-CSF, GM-SCF, and IL-4, which is involved in suppression of eosinophil apoptosis, but IL-5 expression was not altered after DP stimulation. DP also elevated the release of IL-6, IL-8, tumor necrosis factor-α (TNF-α) and CCL2, which are anti-apoptotic or survival factors. The secretion of G-CSF, GM-CSF, IL-6, IL-8, and TNF-α due to DP is higher in atopic normal than that in non-atopic normal. In conclusion, DP increases the survival of eosinophils and its mechanism may be associated with cytokine release. These findings may enable elucidation of asthma pathogenesis induced by HDM.

Key Words: Asthma, House dust mite, Eosinophil apoptosis, Cytokine

Asthma is a chronic inflammation in the respiratory organ and characterized by continuous or paroxysmal labored breathing accompanied by wheezing, bronchial constriction and inflammation, and often by attacks of coughing or gasping. (Milián and Diaz, 2004) According to the World Health Organization (WHO), asthma patients are estimated to be more than 300 million and asthma deaths are estimated to be 300,000 people each year. House dust mite (HDM) is the most important allergen of asthma (Gaffin and Phipatanakul, 2009; Holgate, 2008). HDM allergens include *Dermatophagoides pteronyssinus* (DP) and *Dermatophagoides farina* (DF), which have a deep relationship with the development of asthma (Milián and Diaz, 2004, Lee et al., 2012). Asthma is classified as atopic and non-atopic asthma, depending on the presence of allergen-specific IgE in serum of asthmatic subjects (Corrigan, 2004). The presence of HDM IgE is important in normal subjects as well as in asthmatic subjects as our previous report. (Kim et al., 2013). Eosinophils are associated with allergic diseases such as asthma and allergic rhinitis. Survival of eosinophils is important in pathogenesis of allergic diseases and cytokines secreted from eosinophils are associated with viability and chemotaxis of leucocytes (Filipović and Cekić, 2001). In this study, we introduced the concept of secretosome based on the immunological mechanism of eosinophils and analyzed the effects of HDM on eosinophils.

This study was approved by the Institutional Review Board of Eulji University for normal volunteers and by the Institutional Review Board of Konyang University for asthmatic subjects. All participants in this study gave their written informed consent. Serum was loaded into a Phar-

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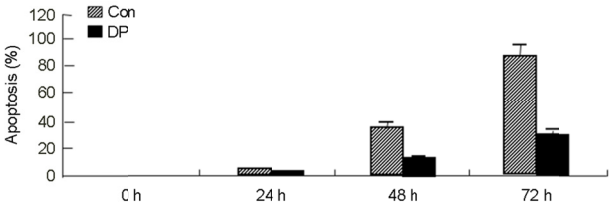

Fig. 1. DP inhibits constitutive apoptosis of asthmatic neutrophils. Eosinophils were isolated from asthmatic peripheral blood (n=3) and then incubated for 24 h, 48 h, and 72 h in the absence (Con) and presence of DP (10 μg/ml). Eosinophil apoptosis was analyzed by measuring the binding of annexin V-FITC and PI. Apoptosis was analyzed by measuring the binding of annexin V-FITC and PI. Data are presented relative to the control, which was set at 100% as the means ± SD.

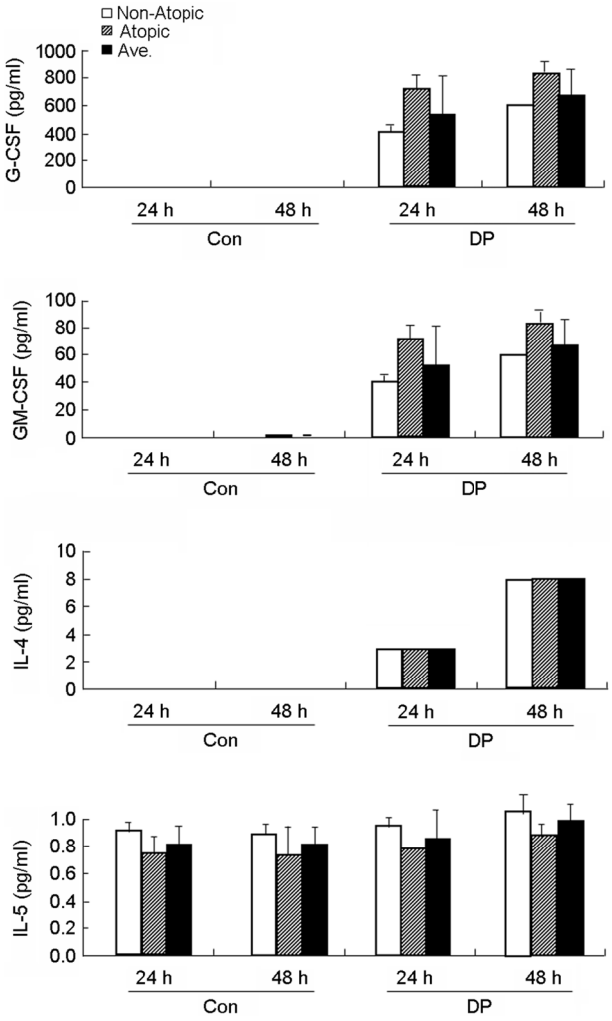

Fig. 2. DP increases the secretion of G-CSF, GM-CSF, IL-4, and IL-5 in non-atopic and atopic normal eosinophils. Eosinophils from peripheral blood of non-atopic and atopic normal (n=2) were incubated with or without 10 μg/ml of DP for 24 h and 48 h. The supernatant was collected and the concentration of G-CSF, GM-CSF, IL-4, and IL-5 in the supernatant was determined by cytokine bioassay using the Luminex 200 system. Data are expressed as the means ± SD.

macia Unicap 100 system (Pharmacia Unicap, Uppsala, Sweden). The DP or DF allergen covalently coupled to the cellulose solid-phase ImmunoCap and reacted with the specific IgE in sera of normal subjects. After washing, enzyme-labeled antibodies against IgE were added to form a complex. The IgE concentration was measured by fluorescence. HDM IgE+ is determined by >0.35 KU/L. Human eosinophils were isolated from the heparinized peripheral blood of normal and asthmatics using Ficoll-Hypaque gradient centrifugation and a CD16 microbeads magnetic cell sorting kit (Miltenyi Biotec, Bergisch Gladbach, Germany). The cells were washed after hypotonic lysis to remove erythrocytes and then resuspended at 3×10^6/ml in RPMI 1640 medium with 1% penicillin-streptomycin and 10% FBS. Counting the cells on cytospin revealed that this method routinely yielded greater than 95% eosinophil purity. An annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit (BD Biosciences, San Diego, CA, USA) was used to detect neutrophil apoptosis. Isolated neutrophils were incubated with an FITC-labeled annexin V and propidium iodide (PI) for 15 min at room temperature. Apoptotic neutrophils were analyzed using a FACSCalibur with CellQuest software (BD bioscience) and were determined as the percentage of cells showing annexin V+/PI- and annexin V+/PI+. Eosinophils were incubated in the absence or presence of 10 μg/ml DP for 24 h and 48 h, and the supernatant was then collected after centrifugation. Cytokine concentration in the supernatant was measured by cytokine bioassay using the Luminex 200 system (Komabiotech Inc. Seoul, Korea).

We examined whether DP alters the regulation of eosinophil apoptosis in asthmatic subjects. DP suppressed the constitutive apoptosis of asthmatic eosinophils in a time-dependent manner (Fig. 1). HDM is closely associated with asthma pathogenesis (Holgate, 2008; Gaffin and Phipatanakul 2009). DP may trigger the pathogenesis of asthma and aggravate the clinical features of asthma by blocking eosinophil apoptosis. Secretion by extracellular

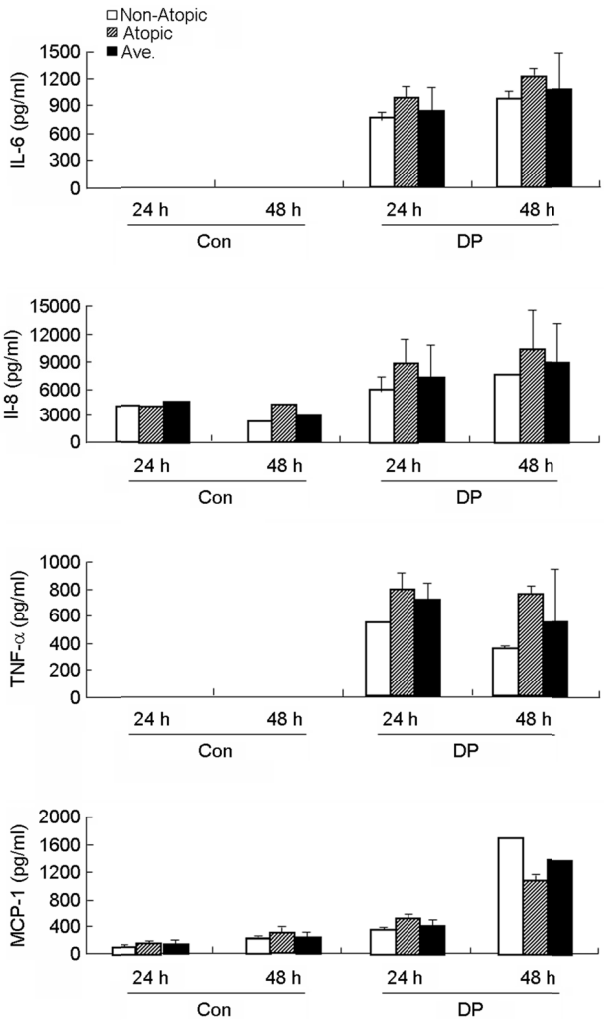

Fig. 3. DP increases the secretion of IL-6, IL-8, TNF-α and MCP-1 in non-atopic and atopic normal eosinophils. Eosinophils from peripheral blood of non-atopic and atopic normal (n=2) were incubated with or without 10 μg/ml of DP for 24 h and 48 h. The supernatant was collected and the concentration of IL-6, IL-8, TNF-α and MCP-1 in the supernatant was determined by cytokine bioassay using the Luminex 200 system. Data are expressed as the means ± SD.

ligands is an crucial step in regulation of eosinophil apoptosis. Therefore, we examined the release of G-CSF, GM-CSF, IL-4, and IL-5, which are survival factors of eosinophils. Because exposure of normal subjects to HDM is essential to the pathogenesis and therapy of asthma, the subjects were classified by HDM-specific IgE (Kim et al., 2013). DP increased the secretion of G-CSF and GM-CSF when compared to the control (Fig. 2). Although IL-4 secretion increased after DP treatment, the concentration was lower than 10 pg/ml. DP has no effect on release of IL-5. Neutrophils are considered important pathogenic factors and a valuable target in asthma treatment (Louis and Djukanovic 2006; Berry et al., 2007). DP induced the secretion of IL-6, IL-8, TNF-α and MCP-1, which is anti-apoptotic factor of neutrophils, despite of different increase of cytokine due to DP (Simon, 2003; Luo and Loison, 2008; Yang et al., 2012) (Fig. 3). These results indicate that DP may regulate eosinophil apoptosis by autocrine secretion and is associated with the plasticity of neutrophil apoptosis. In addition, atopic normal subjects show higher secretion of G-CSF, GM-CSF, IL-6, IL-8, and TNF-α induced by DP than non-atopic normal subjects (Figs. 2 and 3). Exposure of the subjects to HDM is closely involved in cytokine secretion due to HDM. Although the concise anti-apoptotic mechanism and cytokine secretion due to DP was not identified in this study, these results may contribute to a better understanding of the regulation of constitutive eosinophil apoptosis and cytokine secretion due to HDM in normal and asthmatic states.

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REFERENCES

Berry M, Morgan A, Shaw DE, Parker D, Green R, Brightling C, Bradding P, Wardlaw AJ, Pavord ID. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. Thorax 2007. 62: 1043-1049.

Corrigan C. Mechanisms of intrinsic asthma. Curr Opin Allergy Clin Immunol. 2004. 4: 53-56.

Filipović M, Cekić S. The role of eosinophils in asthma. Medicine and Biology. 2001. 8: 6-10.

Gaffin JM, Phipatanakul W. The role of indoor allergens in the development of asthma. Curr Opin Allergy Clin Immunol. 2009. 9: 128-135.

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

Kim IS, Kim MJ, Kim DH, Choi E, Lee JS. Different anti-apoptotic effects of normal and asthmatic serum on normal

eosinophil apoptosis depending on house dust mite-specific IgE. Mol Biol Rep. 2013. 40: 5875-5881.

Lee JS, Yang EJ, Kim IS. The house dust mite allergen, *Dermatophagoides pteronyssinus* suppresses the chemotactic activity of human monocytes. J Exp Biomed Sci. 2012. 18: 435-437.

Luo HR, Loison F. Constitutive neutrophil apoptosis: mechanisms and regulation. Am J Hematol. 2008. 83: 288-295.

Louis R, Djukanovic R. Is the neutrophil a worthy target in severe asthma and chronic obstructive pulmonary disease. Clin Exp Allergy. 2006. 36: 563-567.

Milián E, Diaz AM. Allergy to House Dust Mites and Asthma. P R Health Sci J. 2004. 23: 47-57.

Simon HU. Neutrophil apoptosis pathways and their modifications in inflammation. Immunol Rev. 2003. 193: 101-110.

Yang EJ, Choi E, Ko J, Kim DH, Lee JS, Kim IS. Differential effect of CCL2 on constitutive neutrophil apoptosis between normal and asthmatic subjects. J Cell Physiol. 2012. 227: 2567-2577.