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The Inhibitory Effects of KRGE on Mixed Granulocytic Asthma due to Der p 38

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Abstract

Asthma is a chronic inflammatory airway disease triggered by allergens including house dust mite, food and environmental factors. Korean red ginseng extract (KRGE) has anti-inflammatory effects in asthma, atopic dermatitis and allergic rhinitis. This study aimed to evaluate the suppressive effects of KRGE on mixed-granulocytic asthma induced by Der p 38. In mixed granulocytic asthma-like the mice due to Der p 38, KRGE administration reduced the infiltration of neutrophils and eosinophils in the lungs. In addition, KRGE suppressed the levels of Th1, Th2 and Th17 cytokines in bronchoalveolar lavage fluid (BALF) and reduced serum IgE level. In BEAS-2B cells, Der p 38 increased the secretion of inflammatory cytokines such as interleukin (IL)-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1), which was suppressed by pretreatment with KRGE. Neutrophil apoptosis was delayed by Der p 38 and it was inhibited by KRGE pretreatment. The pro-apoptotic effects of KRGE on neutrophils were closely associated with the activation of caspase 9 and caspase 3, and a decrease in the B-cell leukemia/lymphoma 2 protein (BCL-2)/BCL-2-associated X protein (BAX) expression ratio. These results suggest that KRGE has anti-inflammatory effect in asthma, especially mixed granulocytic asthma, and serves as a potential therapeutic alternative for alleviating mixed granulocytic asthma.

Keywords: Asthma, Korean Red Ginseng Extract, Der p 38, Neutrophil, Cytokine

Major Classifications: Food science, Food Healthy Food, Health management

1. Introduction

Asthma is a chronic inflammatory disease of the airways leading to cough, wheeze, shortness of breath, chest tightness, and mucus over-production (Mims, 2015; Svenningsen & Nair, 2017). Patients with asthma can be categorized into four unique phenotypes, namely eosinophilic, neutrophilic, mixed granulocytic, and paucigranulocytic asthma, based on the analysis of the inflammatory cell count in the sputum (Xiao et al., 2022). In addition, endotypes of asthma are broadly regarded as type 2 (T2) high or T2-low (Kuruvilla et al., 2019). T2-low asthma shows neutrophilic inflammation, which is deeply related to ashtma severity (Ricciardolo et al., 2021; Ray & Kolls, 2017). House dust mite (HDM) is the major allergens affecting allergy pathogenesis (Kim et al., 2013). Der p 38, a component of HDM, is recently identified as a unique allergen that induces the infiltration of neutrophils and eosinophils in a mouse model (Kim & Lee, 2016; Hong et al., 2021). Furthermore, Der p 38 upregulates expression of

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cytokines such as interleukin (IL)-6, IL-8, and monocyte chemoattractant protein-1 (MCP)-1 inhibiting neutrophil apoptosis (Lee, 2022; Lee, 2023).

Korean ginseng has been traditionally used for several millennia in Asian countries, including Korea, China, and Japan, as a therapeutic agent for allergic diseases (So et al., 2018). Korean red ginseng extract (KRGE) reduced IL-5 and increased IL-10 in patients with allergic rhinitis (Jung et al., 2021). Also, KRGE is useful in the management of atopic dermatitis by decreasing the release of TNF-α, IL-1β, IL-6, and IL-8 (Kee et al., 2017). KRGE exerts its therapeutic effects against atopic dermatitis by inhibiting the T helper 2 (Th2)-mediated inflammation and reducing the itching sensation. The proactive systemic administration of KRGE may effectively inhibit early allergic sensitization and suppress Th2 response (Sumiyoshi et al., 2010; Lee et al., 2017). KRGE may contribute to its anti-inflammatory effects by improving antioxidant defense (Park et al, 2020).

Although the effects of KRGE on airway hyper-responsiveness (AHR) and airway inflammation in eosinophilic asthma has been studied, its effect on mixed granulocytic asthma has not yet been conducted and remains unclear (Jeong et al, 2010; Bae et al, 2021). In this study, we investigated the effects of KRGE on mixed-granulocytic asthma *in vitro* and *in vivo*.

2. Materials and Methods

2.1. Reagent

DMEM/F12, RPMI 1640 and FBS were purchased from Gibco™ (Waltham, MA, USA). The Korean Red Ginseng extract used in this research was obtained from the Korea Plant Extract Bank at the Korea Research Institute of Bioscience and Biotechnology (Daejeon, Korea). Cleaved caspase 3, cleaved caspase 9, BAX and BCL-2 antibodies were purchased from Cell Signaling Technology (Beverly, MA). Antibodies against caspase 3, caspase 9, and ERK2 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

2.2. Preparation of Recombinant Der p 38 Protein

Briefly, the mature Der p 38 gene (aa 21-150, GenBank accession number MT273069.1) was cloned into a pETDuet-1 expression vector, and recombinant Der p 38 protein tagged with His was expressed and isolated using a nickel column (Merck Millipore, Darmstadt, Germany) as described in our previous paper (Jeon et al., 2021).

2.3. Cell Culture

Human lung epithelial BEAS-2B cells were cultured in Dulbecco's modified Eagle's medium, supplemented with 10% heated-inactivated fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL). The cells were maintained at sub-confluence in a 5% CO_2 incubator at 37℃. BEAS-2B cells were cultured at a density of $5x10^5$ cells per well in a 6 well plate.

2.4. ELISA

To measure cytokine level, BEAS-2B cells were treated with the Der p 38 (10 μg/mL) or/and KRGE (20 μg/mL) for 48 h, after which the supernatant was collected by centrifugation. The concentration of interleukin (IL)-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1) in the supernatant was measured with a sandwich enzyme-linked immunosorbent assay (ELISA) using OptEIA™ Set human IL-6 (555220), IL-8 (555244), and MCP-1 (555179) (BD Biosciences, San Diego, CA, USA) according to the manufacturer's instructions. ELISA kit for mouse IgE (555248) ELISA Kit (BD Biosciences) was used to measure immunoglobulins in the mice sera. Absorbance was measured at 450 nm using an automated microplate reader (Thermofisher scientific, Waltham, MA, USA).

2.5. Neutrophil Isolation and Western Blotting

Heparinized peripheral blood (15 mL) was collected fro m healthy subjects (n=3). After Ficoll-Hypaque (GE Health care, Chicago, IL, USA) gradient centrifugation, neutrophil s were separated using the CD16 microbeads magnetic cell sorting kit (Multenyi Biotec, Bergisch, Gladbach, German y). After incubating with Der p 38 for 48 h, the cells were h arvested and lysed in lysis buffer (TransLab, Daejeon, Kore a) and centrifugated at 12,000 × g for 15 min at 4℃. The su pernatant was collected as total lysate. Protein concentratio n of the lysate was measured using a protein assay kit (Ther mo Scientific). Proteins (40 μg/lane) were separated on 12% SDS-PAGE and transferred to nitrocellulose membrane. T he membrane was incubated with primary antibodies for 1 h at RT (1:1,000). After three washes with TBS-T, it was in cubated with secondary antibodies (1:3,000) for 1 h at RT, and was developed using the iBright CL750 Imaging Syste m (A44116, Thermo scientific, MA, USA).

2.6. Detection of Apoptosis

Neutrophils and eosinophils treated with Der p 38 or/and KRGE for 24 h were incubated with FITC-labeled annexin V and propidium iodide (PI) (BD Biosciences) for 15 min at room temperature. The stained cells were analyzed by RF-

500 (Sysmex Corporation, Kobe, Japan) and were classified as early apoptotic cells, late apoptotic cells, necrotic cells, and viable cells. Early and late apoptotic cells (annexin V+/PI- and annexin V+/PI+ cells, respectively) are considered apoptotic cells, and the percentage of the cells was evaluated.

2.7. Asthma Induction by Der p 38 in Mice

Six-week-old female C57BL/6J mice were maintained in a specific pathogen-free (SPF) facility. Each group (n=5) was immunized by the intraperitoneal (IP) injection of PBS or Der p 38 (100 μg/50 μL). The groups were administered intranasal (IN) injection of PBS or Der p 38 (50 μg/50 μL) from days 21 to 28 after the first sensitization. The PBS-treated group was considered as the negative control. The Der p 38-treated group was treated with or without oral injection of 50 mg/kg of KRGE. KRGE was dissolved PBS, and then was diluted to less than 1/100 with PBS. All animal experiments used in this study were under a protocol approved by the Institutional Anima Care and Use Committee of the Eulji University (EUIACUC21-24).

2.8. Collection of Bronchoalveolar Lavage Fluid (BALF)

BALF was collected three times by lung lavage via the trachea with 1 mL of PBS. The BALF were centrifuged, and the cells in the BALF were resuspended in 100 μL of PBS for total cell and differential counts. The cells suspended in PBS were attached to a slide by cytospinning (Hanil Scientific Inc, Gimpo, Korea) and stained with a Diff-Quick Kit (Sysmex Corporation). The ratio of each leukocyte was represented as a percentage.

2.9. Statistical Analysis

All data are presented as the means ± SD. Statistical inter-group differences were analyzed using ANOVA with Tukey's post-hoc test for more than two groups. The SPSS statistical software package (Chicago, IL, USA) was used for statistical evaluation. A p value < 0.05 is considered statistically significant.

3. Results

3.1. KRGE Inhibits the Increase of Neutrophils and Eosinophils in Mixed-granulocytic Asthma-like Mice.

Mixed granulocytic asthma was induced in mice through intraperitoneal (IP) and intranasal (IN) injections of Der p 38 (Fig 1A). Following asthma induction with Der p 38, the total number of cells increased, but KRGE administration inhibited their infiltration (Fig 1B). Neutrophils and eosinophils increased after asthma induction, which was related to development of mixed granulocytic asthma. KRGE treatment significantly reduced the inflammatory cells (p<0.05) (Fig 1C, D). Dexamethasone was used for the positive control.

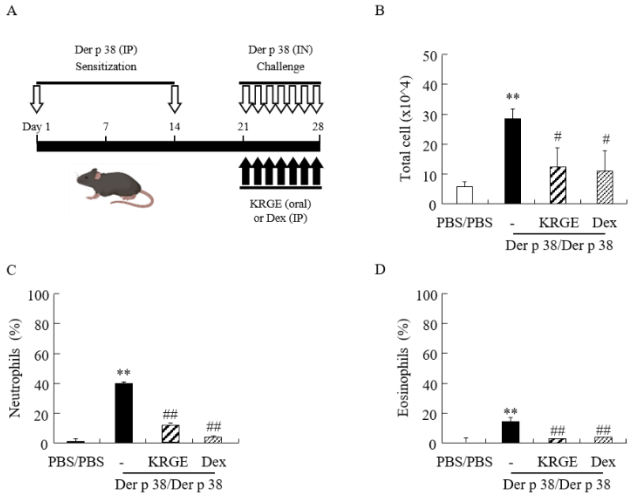

Figure 1: KRGE blocks the infiltration of neutrophils and eosinophils in mixed-granulocytic asthma-like mice (A) Experimental timeline for mixed-granulocytic asthma-like mice induced by Der p 38. (B) Total cell counts in BALF were evaluated. Neutrophils (C) and eosinophils (D) were counted. Data are presented as the means ± SD. **p < 0.01 indicates a significant difference between the control and Der p 38-treated groups. #p < 0.05 and ##p < 0.01 indicates a significant difference between the Der p 38 and Der p 38 + KRGE or dexamethasone-treated groups.

3.2. KRGE Inhibits Th1, Th2 and Th17 Cytokines in Mixed-granulocytic Asthma-like Mice.

Because Der p 38 induces mixed-granulocytic asthma, the alteration of cytokines in BALF was examined. Der p 38 increased Th 1 cytokine, IFN-γ, Th 2 cytokines such as IL-4, IL-5, and IL-13, Th 17 cytokine, IL-17A (Fig 2) in BALF. KRGE reduced IFN-γ, IL-4, IL-5, IL-13, and IL-17A. IL-10 related to inhibition of T cells was not altered by Der p 38 and KRGE. In addition, KRGE suppressed serum total IgE increased by administration of Der p 38 (Fig 3).

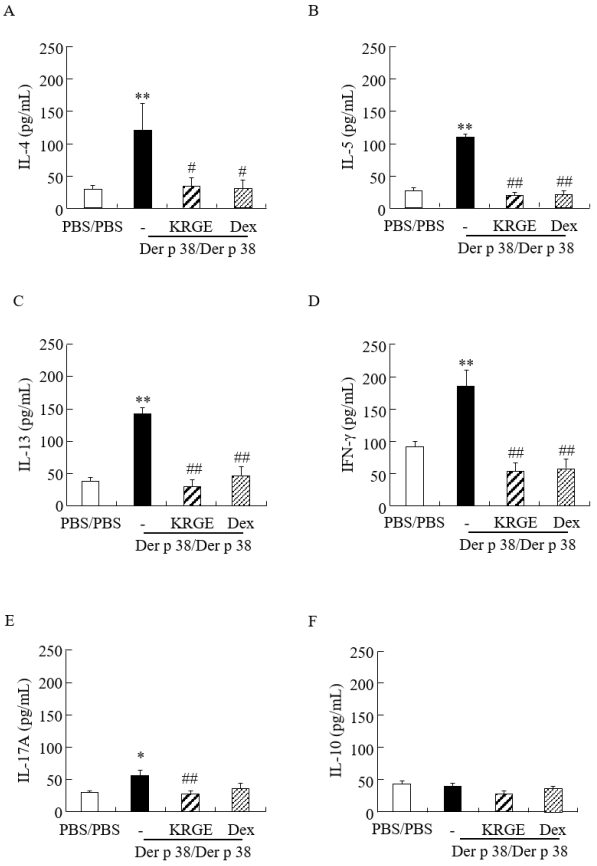

Figure 2: KRGE suppresses the expression of Th1, Th2 and Th17 cytokines. C57BL/6J mice were exposed to Der p 38 (100 μg/50 μL) to induce mixed-granulocytic asthma, and KRGE (20 μg/mL) was administered orally. The concentrations of IL-4 (A), IL-5 (B), IL-13 (C), IFN-γ (D), IL-17A (E), and IL-10 (F) were measured by ELISA in BALF. Data are presented as the means ± SD. $^{**}p < 0.05$ and $^{**}p < 0.01$ indicate a significant difference between groups. $\#\#p < 0.01$ indicates a significant difference between the Der p 38 and Der p 38 + KRGE or dexamethasone-treated groups.

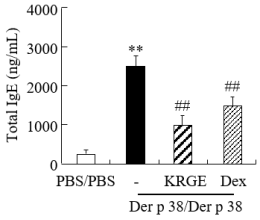

Figure 3: KRGE suppresses the expression of total IgE, IgG1, and IgG2a in mouse serum. C57BL/6J mice were exposed to Der p 38 (100 μg/50 μL) to induce mixed-granulocytic asthma, and KRGE (20 μg/mL) was administered orally. The expressions of Total IgE in the mouse serum were measured. Data are presented as the means ± SD. $^{**}p < 0.01$ indicates a significant difference between the control and Der p 38-treated groups. $\#\#p < 0.01$ indicates a significant difference between the Der p 38 and Der p 38 + KRGE or dexamethasone-treated groups.

3.3. KRGE Inhibits the Secretion of IL-6, IL-8, and MCP-1 by BEAS-2B Cells

To investigate whether KRGE affects the viability of BEAS-2B cells, the cell survival was assessed. The KRGE in concentrations ranging from 10 μg/mL to 20 μg/mL was not effective on cell viability for 48 h (Fig 4A). To investigate the anti-inflammatory effect of the KRGE in detail, we assessed the inhibition of IL-6, IL-8, and MCP-1 secretion related to neutrophil inflammation in BEAS-2B cells. As shown in Fig 4B, the IL-6, IL-8, and MCP-1 levels were elevated by Der p 38, and KRGE reduced the expression of these cytokines. These results suggest that the KRGE acts as a vital suppressor of neutrophil survival and activation induced by Der p 38.

3.4. KRGE Suppresses the Anti-apoptotic Effect of Der p 38 on Neutrophils

Since KRGE suppresses the levels of IL-6, IL-8, and MCP-1 due to Der p 38, we investigated whether it alters neutrophil apoptosis. As shown in Fig 5A, Der p 38 significantly inhibited the spontaneous apoptosis of neutrophils. Co-treatment with Der p 38 and KRGE blocked the anti-apoptotic effect of Der p 38 on neutrophils. Neutrophils undergo constitutive apoptosis through the activation of caspase 9 and caspase 3 after separating from peripheral blood. Der p 38 delayed neutrophil apoptosis by blocking the activation of caspase 9 and caspase 3, while KRGE restored the inhibition of both proteins (Fig 5B, C). Additionally, Der p 38 increased the ration of B-cell leukemia/lymphoma 2 protein (BCL2) to BCL-2-associated protein X (BAX), and KRGE suppressed this increase (Fig 5D-F).

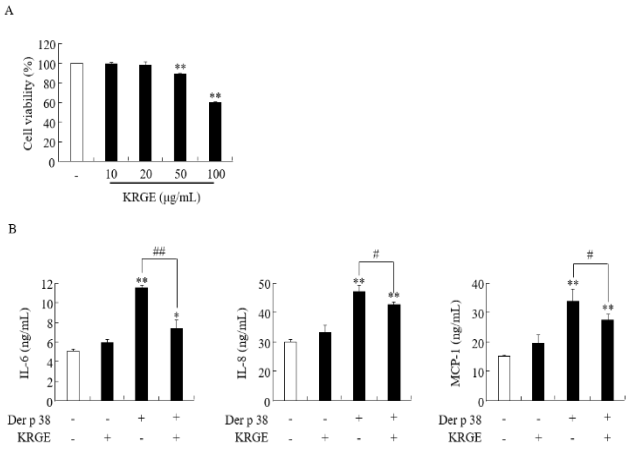

Figure 4: KRGE inhibits the secretion of IL-6, IL-8, and MCP-1 in BEAS-2B cells. (A) BEAS-2B cells was incubated with the KRGE at a concentration of 10, 20, 50, and 100 μg/mL for 48 h. The data was expressed as the relative ration to the absorbance of the untreated cells, which was set at 100%. (B) The cells were treated with Der p 38 (10 μg/mL) or KRGE extract (20 μg/mL), or co-treated with Der p 38 and KRGE. The concentration of IL-6, IL-8, and MCP-1 was measured with ELISA in the cell supernatant. Data are expressed as the means ± SD; ***p* < 0.01 indicate a significant difference between the control and Der p 38-treated groups. #*p* < 0.05 and ##*p* < 0.01 indicates a significant difference between the Der p 38 and Der p 38 + KRGE-treated group.

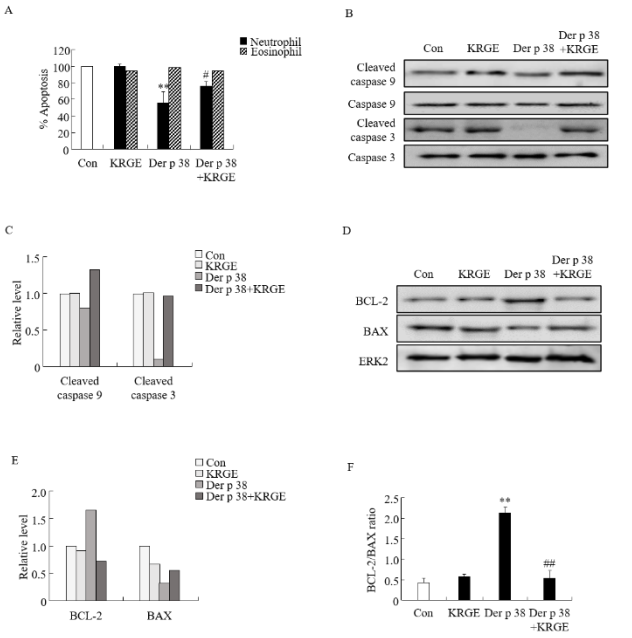

Figure 5: KRGE inhibits the anti-apoptotic effect of Der p 38 on neutrophils. (A) Neutrophils were isolated and incubated for 24 h in the absence or presence of Der p 38 (10 μg/mL) or/and KRGE (20 μg/mL). Apoptotic cells were evaluated using annexin-PI staining. Control was set to be 100% (n=3). (B and D) The expressions of cleaved caspase 9, caspase 3, BCL-2, and BAX were detected by western blotting. (C and E) Densitometric data from B and D were presented relative to the negative control set at 1. (F) Ratio of BCL-2/BAX were calculated by densitometric analysis. Data are presented as the means ± SD; ***p* < 0.01 indicate a significant difference between the control and Der p 38-treated groups. #*p* < 0.05 and ##*p* < 0.01 indicate a significant difference between the Der p 38 and Der p 38 + KRGE-treated groups.

4. Discussion

Asthma phenotypes are currently classified into type 2 (T2)-high and T2 low asthma (Maison et al., 2022). T2-low asthma (non-T2) is often associated with a previous eosinophilic phenotype, which typically responds well to anti-inflammatory treatment (Ntontsi et al., 2017). In contrast, mixed-granulocytic asthma, a subtype involving both neutrophils and eosinophils, tends to be resistant to steroid therapy, highlighting the urgent need for novel therapeutic options (Pignatti et al., 2022). Neutrophil apoptosis regulation is a critical factor in T2-low asthma (Ricciardolo et al., 2021; Lee, 2014). In a prior study, we identified Der p 38 as a key contributor to mixed granulocytic asthma, facilitating the establishment of the asthma mouse model. Notably, Der p 38 influences neutrophil apoptosis, a process central to the development of mixed granulocytic phenotype, unlike other components of house dust mite (Hong et al., 2021; Lee, 2023). Neutrophils play a crucial role in asthma, contributing to airway remodeling and mucus hypersecretion, which lead to airway narrowing (Yamasaki et al., 2021). KRGE inhibited the infiltration of neutrophils and eosinophils due to Der p 38 (Fig. 1). Although it suppresses the movement of inflammatory cells into the lung, its effects on mucus secretion and airway remodeling remain unknown and require further investigation.

Korean red ginseng is traditionally used as an important herbal medicine in Far East Asia (Lee et al., 2015). An earlier investigation, it prevented ovalbumin (OVA)-induced morphological changes in the pulmonary system by restoring the balance of Th1/Th2 cytokines including transcription factors, and blocked inflammatory pathways such as NF-κB/COX-2 and PGE2 (Lee et al., 2021). Similarly, KRGE has shown anti-asthmatic effects in an OVA-sensitized mouse model (Lim et al., 2015). Additionally, KRGE inhibits the activation of mitogen-activated protein kinases (MAPKs) and transcription factors, such as NF-κB and c-Fos in lung inflammatory responses (Lee et al., 2018). In this study, we focus on the

cytokines associated with neutrophils in mixed-granulocytic asthma. Our results indicate that the KRGE suppresses the secretion of cytokines, including IL-6, IL-8, and MCP-1 (Fig 4). Moreover, we found that KRGE reduces T1, T2, and Th17 cytokines as well as IgE, indicating that it inhibits the pathogenic mechanism of mixed-granulocytic asthma mice. (Figs 2 and 3). Additionally, KRGE increases IgG1 and IgG2a associated with alleviation of asthmatic symptoms (Supplemental Fig 1).

As shown in Fig 4, the neutrophil apoptosis was delayed by Der p 38; however, KRGE inhibits the suppressive effect. Capase-9 and caspase-3 are activated by downstream of the initiators of apoptosis, which occurs during neutrophil apoptosis (Bram et al., 2008). Der p 38 delayed neutrophil apoptosis by inhibiting the activation of caspase 9 and caspase 3, while KRGE effectively reversed this suppression of both proteins (Fig 5).

In conclusion, this study demonstrates that KRGE inhibits the secretion of Th2 and Th17 cytokines, resulting in reduction of neutrophils and eosinophils in mixed granulocytic asthma-like mice. In addition, it inhibits IL-6, IL-8, and MCP-1, thereby suppressing the delayed neutrophil apoptosis. KRGE may be a promising candidate for treatment or prevention of specific asthma phenotype.

Availability of Data and Materials

All supporting information including table of results and detailed methods are available upon request.

Acknowledgement

none

Conflict of Interest

Authors declare that they have no conflicts of interests.

Reference

Bae, CH., Kim, JS., Nam, W., Kim, H., Kim, J., Nam, B., Park, S., Lee, J., and Sim, J. (2021) Fermented red ginseng alleviates ovalbumin-induced inflammation in mice be suppressing interleukin-4 and immunoglobulin E expression. *Journal of medicinal food. 24*(6), 569-576. http://doi.org/10.1089/jmf.2020.4854.

Bak, MJ., Hong, S-G., Lee, J-W., and Jeong, W-S. (2012) Red ginseng marc oil inhibits iNOS and COX-2 via NFκB and p38 pathways in LPS-stimulated RAW 264.7. *Molecules. 17*(12), 13769-13789. http://doi.org/10.3390/molecules171213769.

Hong, MH., Kashif, A., Kim, G., Park, BS., Lee, NR., Yang, EJ., Mun, JY., Choi, H., Kim, S-H., Kim, HJ., Lee, SJ., Lee, J-S., Hong, YJ., and Kim, IS., (2021) Der p 38 is a bidirectional regulator of eosinophils and neutrophils in allergy. *The journal of immunology. 207*(7), 1735-1746. http://doi.org/10.4049/jimmunol.2001144.

Jeon, Hyang., Kim, G., Kashif, A., Hong, MH., Lee, J-S., Hong, Y., Park, BS., Yang, EJ., and Kim, IS. (2021) Pathogenic mechanism of Der p 38 as a novel allergen homologous to RipA and RipB proteins in atopic dermatitis. *Frontiers in immunology. 12*, 646316. http://doi.org/10.3389/fimmu.2021.646316.

Jeong, YJ., Paeng, JW., Choi, DC., and Lee, BJ. (2010) Effects of Korean red ginseng extracts on airway hyperresponsiveness and inflammation in a murine asthma model. *Journal of asthma, allergy and clinical immunology. 30*(1), 43-49.

Jung, JH., Kang, TK., Oh, JH., Jeong, JU., Ko, KP., and Kim, ST. (2021) The effect of korean red ginseng of symptoms and inflammation in patients with allergic rhinitis. *Ear, nose & throat journal. 100*(5_suppl), 712S-719S. http://doi.org/10.1177/0145561320907172.

Kee, JY., Jeon, YD., Kim, DS., Han, Y-H., Park, J., Youn, D-H., Kim, S-J., Ahn, KS., Um, JY., and Hong, S-H. (2017) Korean red ginseng improves atopic dermatitis-like skin lesions by suppressing expression of proinflammatory cytokines and chemokines in vivo and in vitro. *Journal of ginseng research. 41*(2), 134-143. https://doi.org/10.1016/j.jgr.2016.02.003.

Kim, IS., Kim, EH., Kim, DH., Kim, JS., and Lee, JS. (2013) Effect of house dust mite and CCL2 on S100A8 and S100A9 expression in human monocytes. *Biomedical Science Letters. 19*(4), 344-347.

Kim, IS. And Lee, J-S. (2016) Association of anti-apoptotic mechanism due to house dust mite in neutrophils with protein synthesis and Bad. *Biomedical Science Letters. 22*, 211-214. https://doi.org/10.15616/BSL.2016.22.4.211

Kuruvilla, ME., Lee, F.E-H., and Lee, GB. (2019) Understanding asthma phenotypes, endotypes, and mechanisms of disease. *Clinical Reviews in Allergy & Immunology. 56*(2), 219-233. http://doi.org/10.1007/s12016-018-8712-1.

Lee, HJ. and Cho, SH. (2017) Therapeutic effects of Korean red ginseng extract in a murine model of atopic dermatitis: anti-pruritic and anti-inflammatory mechanism. *Journal of korean medical science. 32*(4), 679-687. https://doi.org/10.3346/jkms.2017.32.4.679

Lee, JH., Min, DS., Lee, CW., Song, KH., Kim, YS., and Kim, HP. (2018) Ginsenosides from Korean red ginseng ameliorate lung inflammatory responses: inhibition of the MAPKs/NK-κB/c-Fos pathways. *Journal of ginseng research. 42*(4), 476-484. https://doi.org/10.1016/j.jgr.2017.05.005.

Lee, JS. (2023) Allergic effects of Der p 38 and Der f 38: A comparison. *Biomedical Science Letters. 29*, 206-9. https://doi.org/10.15616/BSL.2023.29.3.206.

Lee, JS. (2022) Anti-inflammatory effects of neuregulin-1 via the downregulation of IL-6, IL-8, and MCP-1 secretion. Biomedical *Science Letters. 28*, 192-4. https://doi.org/10.15616/BSL.2022.28.3.192.

Lee, JS., Choi, E., Yang, EJ., Lee, NR., Baek, SY., Kim, EJ., and Kim, IS. (2014) Induction of the neutrophil migration in normal subjects due to asthmatic bronchoalveolar lavage fluid (BALF). *Biomedical Science Letters. 20*, 111-116. https://doi.org/10.15616/BSL.2014.20.3.111.

Lee, SM., Bae, BS., Park, H-W., Ahn, N-G., Cho, B-G., Cho, Y-L., and Kwak, Y-S. (2015) Characterization of korean red

ginseng (Panax ginseng Meyer): History, preparation method, and chemical composition. *Journal of Ginseng Research. 39*(4), 384-91. http://doi.org/10.1016/j.jgr.2015.04.009.

Lee, SY., Kim, MH., Kim, S-H., Ahn, T., Kim, S-W., Kwak, Y-S., Cho, I-H., Nah, S-Y., Cho, S-S., Park, KM., Park, D-H., and Bae, C-S. (2021) Korean red ginseng affects ovalbumin-induced asthma by modulating IL-12, IL-4, and IL-6 levels and the NF-κB/COX-2 and PGE2 pathways. *Journal of ginseng research. 45*(4), 482-9. http://doi.org/10.1016/j.jgr.2020.10.001.

Lim, CY., Moon, JM., Kim, BY., Lim, SH., Lee, GS., Yu, HS., and Cho, SI. (2015) Comparative study of korean white ginseng and korean red ginseng on efficacies of OVA-induced asthma model in mice. *Journal of ginseng research. 39*(1), 38-45. http://doi.org/10.1016/j.jgr.2014.07.004.

Maison, N., Omony, Jimmy., Illi, S., Thiele, D., Skevaki, C., Dittrich, A-M., Bahmer, T., Rabe, KF., Weckmann, M., Happle, C., Schaub, B., Meyer, M., Foth, S., Rietschel, E., Renz, H., Hansen, G., Kopp, MV., Mutius, EV., Grychtol, R. et al. (2022) T2-high asthma phenotypes across lifespan. *European respiratory journal. 60*(3), 2102288. http://doi.org/10.1183/13993003.02288-2021.

Mims, J.W. (2015) Asthma: definitions and pathophysiology. *International Forum of Allergy & Rhinology. 5*(Suppl 5), S2–S6. http://doi.org/10.1002/alr.21609.

Ntontsi, P., Loukides, S., Bakakos, P., Kostikas, K., Papatheodorou, G., Papathanassiou, E., Hillas, G., Koulouris N., Papiris, S., and Papaioannou, AI. (2017) Clinical, functional and inflammatory characteristics in patients with paucigranulocytic stable asthma: Comparison with different sputum phenotypes. *Allergy. 11*, 1761–1767. http://doi.org/10.1111/all.13184.

Park, SK., Hyun, SH., In, G., Park, CK., Kwak, YS., Jang, YJ., Kim, B., Kim, JH., and Han, CK. (2020) The antioxidant activities of Korean Red Ginseng (Panax ginseng) and ginsenosides: A systemic review through in vivo and clinical trials. *Journal of Ginseng Research. 45*(1), 41-47. http://doi.org/10.1016/j.jgr.2020.09.006.

Pignatti, P., Vista, D., Zappa, M., Zampogna, E., Saderi, L., Sotgiu, G., and Spanevello, A. (2022) Analysis of patients with asthma and mixed granulocytic inflammatory pattern in sputum. *Respiration. 10*(12), 1121-30. http://doi.org/10.1159/000527125.

Sumiyoshi, M., Sakanaka, M., and Kimura, Y. (2010) Effects of red ginseng extract on allergic reactions to food in Balb/c mice. *Journal of ethnophamacology. 32*(4), 206-212. http://doi.org/10.1016/j.jep.2010.08.012.

Raam, BJV., Drewniak, A., Groenewold, V., K van den Verg, Timo., and Kuijpers, TW. (2008) Agata Drewniak et al. Granulocytic colony-stimulating factor delays neutrophil apoptosis by inhibition of calpains upstream of caspase-3. *Blood. 112*(5), 2046-2054. http://doi.org/10.1182/blood-2008-04-149575.

Ray, A., Kolls, JK. (2017) Neutrophilic inflammation in asthma and association with disease severity. *Trends in Immunology. 38*(12), 942–954. http://doi.org/10.1016/j.it.2017.07.003.

Ricciardolo, F LM., Carriero, V., and Bertolini, F. (2021) Which therapy for non-type(T)2/T2-low asthma. *Journal of personalized medicine. 12*(1), 10. http://doi.org/10.3390/jpm12010010.

So, SH., Lee, JW., Kim, YS., Hyun, SH., and Han, CK. (2018) Red ginseng monograph. *Journal of Ginseng Research. 42*, 549-561. http://doi.org/10.1016/j.jgr.2018.05.002.

Svenningsen, S., and Nair, P. (2017) Asthma endotypes and an overview of targeted therapy for asthma. *Frontier in Medicine. 26*(4), 158. http://doi.org/10.3389/fmed.2017.00158.

Xiao, S., Zhou, Y., Wang, Q., and Yang, D. (2022) Ketamine attenuates airway inflammation via inducing inflammatory cells apoptosis and activating Nrf2 pathway in a mixed-granulocytic murine asthma model. *Drug designs, development, and therapy. 16*, 4411–4428. http://doi.org/10.2147/DDDT.S391010.

Yamasaki, A., Okazaki, R., and Harada, T. (2022) Neutrophils and asthma. *Diagnosis. 12*(5), 1175. http://doi.org/10.3390/diagnostics12051175.