

ORIGINAL ARTICLE

Effects of Angelicin on Ovalbumin (OVA)-Induced Airway Inflammation in a Mouse Model of Asthma

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Abstract—Angelicin, a furocoumarin found in *Psoralea corylifolia* L. fruit, has been reported to have anti-inflammatory activity. The purpose of this study was to determine the protective effects of angelicin on allergic asthma induced by ovalbumin (OVA) in mice. Mice were sensitized to OVA (on days 0 and 14) and challenged with OVA three times (on days 21 to 23). Angelicin (2.5, 5, 10 mg/kg) was given intraperitoneally 1 h before OVA treatment after the initial OVA sensitization. The production of IL-4, IL-5, and IL-13 in BALF and IgE in the serum were measured by ELISA. Lung histological changes were detected by using hematoxylin and eosin (H&E) stain. The results showed that angelicin significantly inhibited inflammatory cells infiltration into the lungs. Histological studies showed that angelicin significantly attenuated OVA-induced lung injury. Meanwhile, treatment of angelicin dose-dependently inhibited OVA-induced the production of IL-4, IL-5, and IL-13 in BALF and IgE in the serum. Furthermore, angelicin was found to inhibit airway hyperresponsiveness and NF- κ B activation. In conclusion, our results suggested that angelicin inhibited allergic airway inflammation and hyperresponsiveness by inhibiting NF- κ B activation.

KEY WORDS: angelicin; OVA; asthma; NF- κ B.

INTRODUCTION

Allergic asthma is an inflammatory disease of the airways which is characterized by chronic inflammation of the airways, bronchial hyperreactivity, inflammatory cells infiltration, and reversible airway obstruction [1]. Animal models and clinical studies in humans suggested that T helper type 2 lymphocytes played critical roles in the pathogenesis of allergic asthma by producing cytokines IL-4, IL-5, and IL-13

[2, 3]. These cytokines lead to the release of IgE and inflammatory cells infiltration [4]. The inflammatory cells release a various of chemical mediators which lead to the development of bronchial hyperreactivity [5]. Furthermore, the release of IgE has been identified as the important mediator that lead to the pathogenesis of allergic asthma [6]. Therefore, inhibition of these cytokines might be a potential therapeutic strategy for the treatment of allergic asthma.

Angelicin, a furocoumarin found in *Psoralea corylifolia* L. fruit, has been reported to have antimicrobial and antitumor effects [7]. Recently, angelicin has been reported to have anti-inflammatory effect. Angelicin has been shown to inhibit inflammatory cytokines TNF- α , IL-6, and IL-1 β production in LPS-stimulated RAW264.7 cells [8]. Angelicin also inhibited LPS-induced NO production in LPS-stimulated macrophages [9]. In addition, angelicin has been reported to have protective effects against LPS-induced acute lung injury in mice [8].

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However, whether angelicin have protective effects against allergic asthma remain unclear. Ovalbumin (OVA)-induced asthma demonstrates many features of airway allergy and allergic asthma that are similar to the human conditions [10]. Therefore, in this study, we investigate the protective effects and mechanism of angelicin on OVA-induced asthma in mice.

MATERIALS AND METHODS

Reagents

Angelicin (purity >98 %) was purchased from the National Institutes for Food and Drug Control (Beijing, China). Dimethyl sulfoxide (DMSO), MTT, and OVA were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Mouse IL-4, IL-5, and IL-13 ELISA kits were purchased from eBioscience (USA). Mouse IgE ELISA kit was obtained from R&D Systems (Minneapolis, MN). Antibodies against NF- κ B, I κ B α , and β -actin were purchased from Cell Signaling Technology (Beverly, MA, USA). All other chemicals were of reagent grade.

Animals

Female BALB/c mice, 18 to 22 g, were purchased from the Center of Experimental Animals of Liaoning Medical College (Liaoning, China). The mice were accustomed to the environment for 7 days. The mice were housed in an animal facility with the temperature of 23 ± 2 °C. The mice were received food and water *ad libitum*. All animal experiments were performed in accordance with the Health's Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health.

Experimental Design and Grouping

Sixty mice were randomly divided into six groups: control group, OVA group, OVA + angelicin (2.5, 5, 10 mg/kg) group, OVA + DEX group. The mice were sensitized with 20 μ g OVA adsorbed in 200 μ l PBS with 1 mg aluminum hydroxide by intraperitoneal injection at days 0 and 14. Then the mice were received 100 μ g OVA adsorbed in 20 μ l PBS by intranasal inhalations at days 21, 22, and 23 once a day. Angelicin (2.5, 5, 10 mg/kg) was given intraperitoneally 1 h before OVA treatment after the initial OVA sensitization. The mice were sacrificed 24 h after the last challenge.

Histopathologic Studies of Lung

Twenty-four hours after the last OVA treatment, the lungs were collected and fixed with 10 % formaldehyde. Then the lung tissues were dehydrated, embedded in paraffin, and sliced. Then the sections were stained with hematoxylin and eosin (H&E) for general morphology.

Collection of BALF and Cell Counting

Twenty-four hours after the last OVA treatment, the mice were sacrificed and the BALF were obtained by intratracheal instillation with 0.8 ml cold PBS. Then the BALF samples were centrifuged and the supernatants were used to analysis the levels of cytokines. The cell pellets were suspended with PBS for cell counts. Neutrophils, macrophages, eosinophils, and lymphocytes in BALF were stained with the Kwik-Diff staining set (Thermo).

ELISA Assay

The levels of the cytokines including IL-4, IL-5, and IL-13 in the BALF and IgE in the serum were measured by using ELISA kits according to the manufacturer's instructions.

Measurement of Airway Hyperresponsiveness

Twenty-four hours after the last OVA treatment, airway hyperresponsiveness (AHR) was measured using non-invasive whole-body plethysmography (Buxco, Sharon, CT) as described previously [11]. AHR was expressed as an enhanced minute pause (Penh), a calculated value that reflects pulmonary resistance. The results are expressed as the percentage increase in Penh over the baseline following challenge performed with each concentration of methacholine, where the baseline Penh (after PBS challenge) is expressed as 100 %.

Western Blot Analysis

Total proteins from lung tissues were extracted using T-PER Tissue Protein Extraction Reagent Kit (Pierce, USA) according to the manufacturer's instructions. Equal amounts (40 μ g) of proteins were separated on 12 % SDS-PAGE gel and transferred onto nitrocellulose membranes. The membranes were blocked with blocking solutions (5 % skim milk) for 2 h at room temperature. Then the membranes probed with primary antibodies: NF- κ B, p-NF- κ B, I κ B α , p-I κ B α , and β -actin at 4 °C overnight. Finally, the membranes were incubated with HRP-conjugated

secondary antibody and developed using an enhanced chemiluminescence kit (Thermo, USA).

Statistical Analysis

All values were presented as mean $\pm$ S.E.M. Significant differences were evaluated by ANOVA followed by Bonferroni post hoc multiple comparison test using the SPSS software 18.0. *P* values of 0.05 or less were considered statistically significant.

RESULTS

Effects of Angelicin on Inflammatory Cell Count in the BALF of OVA-Induced Asthma Model

Effects of angelicin on inflammatory cell count in the BALF were measured in this study. As shown in Fig. 1, OVA-challenged mice displayed significant increases in the number of inflammatory cells (neutrophils, eosinophils, macrophages, and lymphocytes) in BALF compared to the normal control mice. However, treatment of angelicin or DEX significantly inhibited OVA-induced inflammatory cells infiltration in BALF (Fig. 1).

Angelicin Decreased the Levels of Th2 Cytokines in the BALF

Th2 cytokines have been reported to play important roles in the pathogenesis of asthma. In this study, the

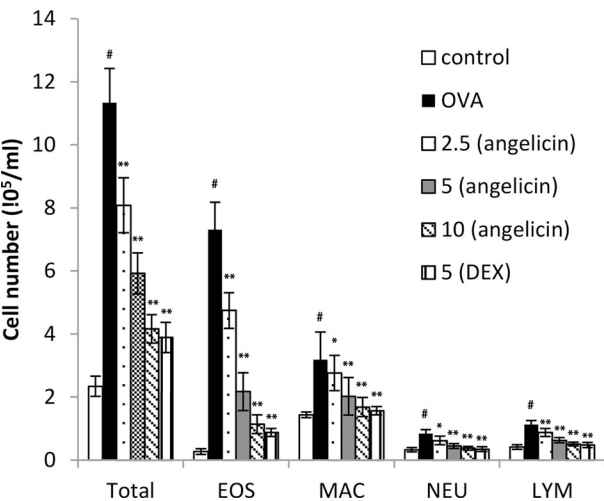


Fig. 1. Effects of angelicin on OVA-induced inflammatory cell count in the BALF. The values presented are the means $\pm$ SEM of three independent experiments. #*p* < 0.05 vs. control group; **p* < 0.05, ***p* < 0.01 vs. OVA group.

production of IL-4, IL-5, and IL-13 in BALF were detected. The results showed that IL-4, IL-5, and IL-13 levels in the BALF were elevated in OVA-challenged mice *versus* the control mice. However, angelicin dose-dependently suppressed OVA-induced IL-4, IL-5, and IL-13 production (Fig. 2).

Angelicin Inhibited OVA-Specific IgE in the Serum of OVA-Induced Asthma Model

The level of serum IgE was measured by ELISA in this study. As shown in Fig. 3, the results showed that

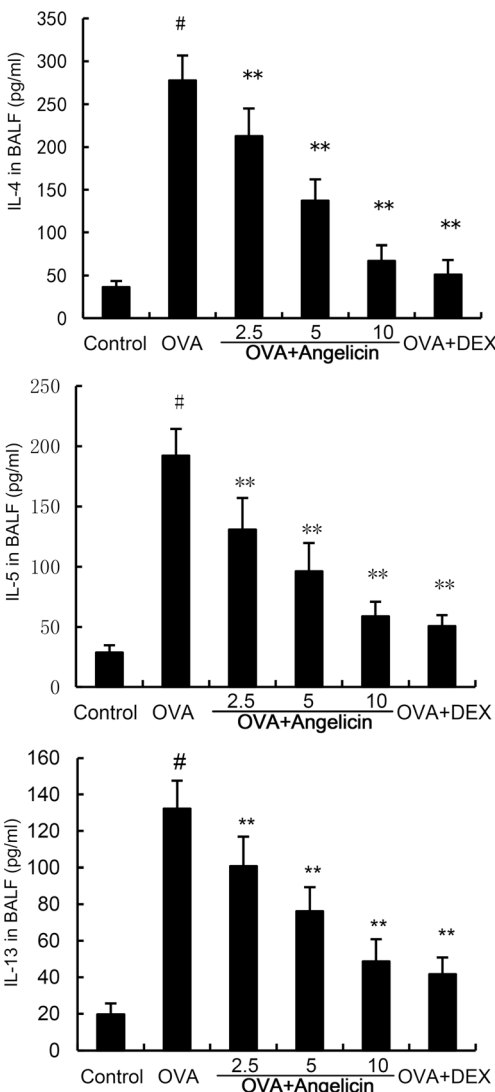


Fig. 2. Effects of angelicin on OVA-induced IL-4, IL-5, and IL-13 productions in BALF were detected by ELISA. The values presented are the means $\pm$ SEM of three independent experiments. #*p* < 0.05 vs. control group; **p* < 0.05, ***p* < 0.01 vs. OVA group.

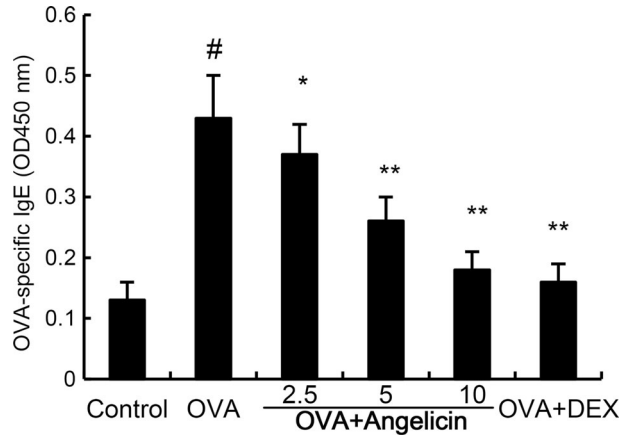


Fig. 3. Effects of angelicin on OVA-induced IgE production. The level of IgE in the serum was measured by ELISA. The values presented are the means $\pm$ SEM of three independent experiments. # p < 0.05 vs. control group; * p < 0.05, ** p < 0.01 vs. OVA group.

serum IgE level in BALF was elevated in OVA-challenged mice *versus* the control mice. However, angelicin dose-dependently suppressed OVA-induced serum IgE production.

Effects of Angelicin on OVA-Mediated Lung Histopathologic Changes of OVA-Induced Asthma Model

The effects of angelicin on OVA-induced lung histological changes were assessed by H&E staining. The results showed that lung tissues from OVA group were significantly damaged, including inflammatory

cell infiltration in the peribronchial and perivascular areas and airway epithelial thickening. However, treatment of angelicin markedly ameliorated OVA-induced lung injury (Fig. 4).

Angelicin Reduced OVA-Induced Airway Hyperresponsiveness

The effects of angelicin on AHR were measured using noninvasive whole-body plethysmography (Buxco, Sharon, CT) in this study. As shown in Fig. 5, the Penh value of OVA-challenged mice increased significantly than the control group. Treatment of angelicin dose-dependently inhibited Penh value induced by OVA (Fig. 5).

Effects of Angelicin on OVA-Induced NF- κ B Activation

The effects of angelicin on OVA-induced NF- κ B activation were detected by western blot analysis in this study. As shown in Fig. 6, OVA significantly induced NF- κ B activation. However, treatment of angelicin inhibited OVA-induced NF- κ B activation in a dose-dependent manner.

DISCUSSION

In the present study, we investigated the protective effects of angelicin on OVA-induced asthma. Our results showed that angelicin had protective effects against OVA-induced asthma. Angelicin attenuated OVA-induced lung injury, airway hyperresponsiveness, and the levels of OVA-specific IgE,

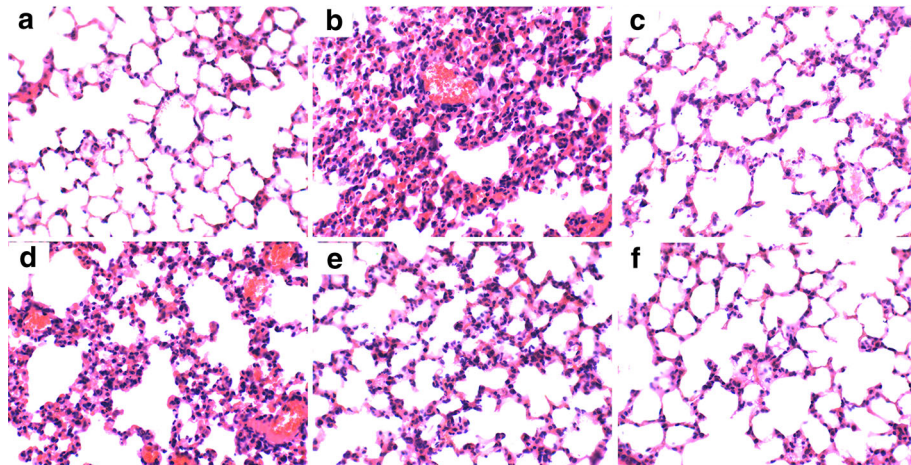


Fig. 4. Effects of angelicin on histopathological changes in lung tissues in OVA-induced asthma. Lung tissues from each experimental group were processed for histological evaluation. Representative histological changes of lung obtained from mice of different groups. **a** Control group, **b** OVA group, **c** OVA + DEX group, **d** OVA + angelicin (2.5 mg/kg) group, **e** OVA + angelicin (5 mg/kg) group, **f** OVA + angelicin (10 mg/kg) group (hematoxylin and eosin staining, magnification 200 $\times$).

Effects of Angelicin on Ovalbumin (OVA)-Induced Airway

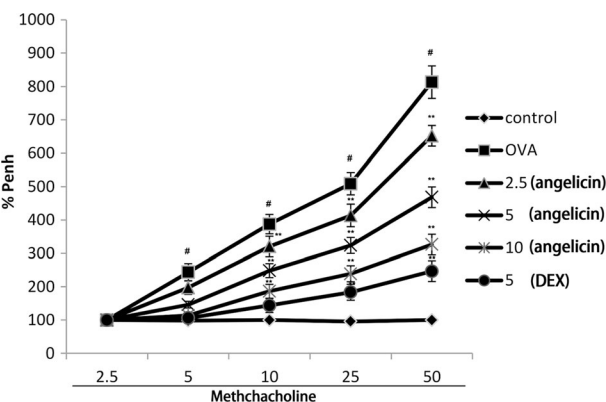


Fig. 5. Angelicin alleviated OVA-induced airway hyperresponsiveness. The effects of angelicin on AHR were measured using noninvasive whole-body plethysmography (Buxco, Sharon, CT). The values presented are the means $\pm$ SEM of three independent experiments. [#] $p < 0.05$ vs. control group; * $p < 0.05$, ** $p < 0.01$ vs. OVA group.

IL-4, IL-5, and IL-13. Angelicin protected against OVA-induced asthma by inhibiting NF- κ B activation.

OVA-induced asthma is characterized by the infiltration of inflammatory cells (neutrophils, eosinophils, macrophages, and lymphocytes) in the lung [12]. Studies showed that the infiltration of inflammatory cells played important roles in the pathogenesis of allergic asthma by releasing cytotoxic granule proteins [13–15]. In this study, our results showed that angelicin significantly inhibited OVA-induced inflammatory cells infiltration. Furthermore, histological analysis showed that angelicin attenuated OVA-induced lung tissue injury, suggesting angelicin had protective effects against OVA-induced asthma. Th2 cytokines such as IL-4, IL-5, and IL-13 have been reported to play critical roles in the induction of inflammatory cells infiltration into the lungs [16, 17]. IL-4 causes B cell maturation and synthesizes IgE [18, 19]. IL-5 plays a critical role in the differentiation and recruitment of eosinophils [20, 21]. IL-13 could induce asthma symptoms such as airway inflammation and AHR [22–24]. Elevated IL-4, IL-5, and IL-13 levels were observed in patients with

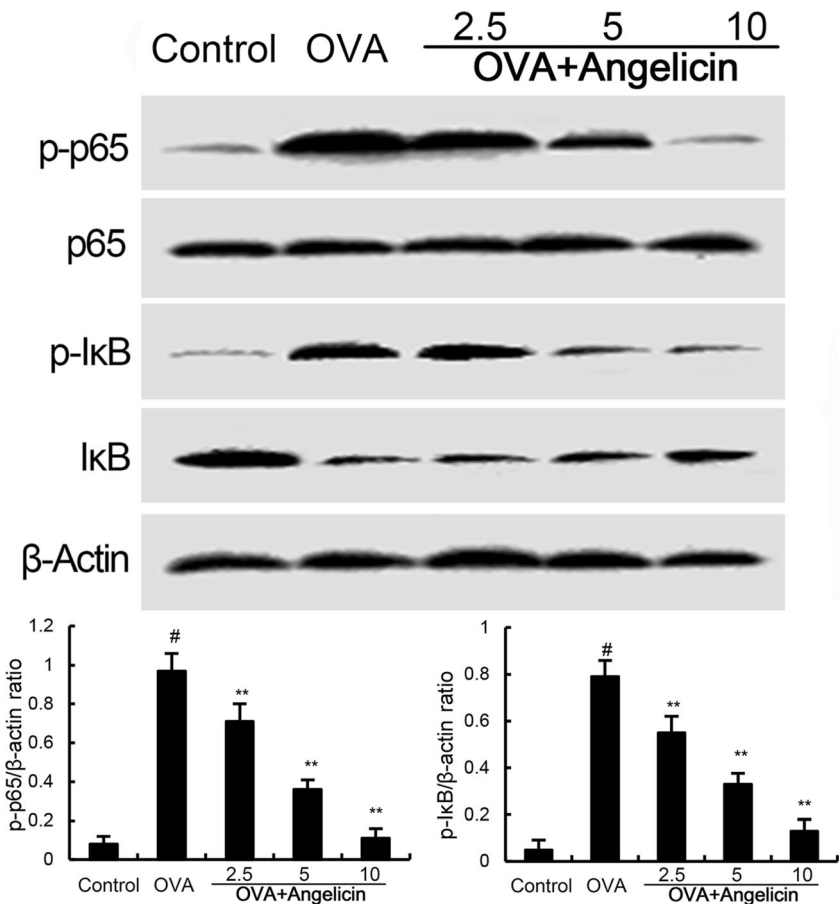


Fig. 6. Effects of angelicin on OVA-induced NF- κ B activation. The expression of NF- κ B and I κ B α was detected by western blotting. The values presented are the means $\pm$ SEM of three independent experiments. [#] $p < 0.05$ vs. control group; * $p < 0.05$, ** $p < 0.01$ vs. OVA group.

asthma [25, 26]. In this study, we found that angelicin dose-dependently inhibited OVA-induced IL-4, IL-5, and IL-13 production. In addition, OVA-induced IgE and AHR were also attenuated by treatment of angelicin.

NF- κ B, an essential transcription factor, has been reported to play critical roles in the regulation of cytokines production [27]. Under normal physiological conditions, NF- κ B is sequestered in the cytoplasm and linked to I κ B protein [28]. Once stimulated, I κ B detaches from NF- κ B and NF- κ B translocation into the nucleus to regulate inflammatory cytokine gene transcription [29]. Previous studies showed that activation of NF- κ B was involved in allergic airway inflammation both in patients or animal model of asthma [30]. To investigate the protective mechanism of angelicin against OVA-induced asthma, the effects of angelicin on OVA-induced NF- κ B activation were detected. The results showed that angelicin inhibited OVA-induced NF- κ B activation in a dose-dependent manner.

In conclusion, our results demonstrated that angelicin had protective effects against OVA-induced asthma in mice. Angelicin protected against OVA-induced asthma by inhibiting NF- κ B activation. These evidences suggested that angelicin had a potential application to treat OVA-induced asthma.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflict of interest.

REFERENCES

- Bousquet, J., P.K. Jeffery, W.W. Busse, M. Johnson, and A.M. Vignola. 2000. Asthma. From bronchoconstriction to airways inflammation and remodeling. *American Journal of Respiratory and Critical Care Medicine* 161(5): 1720–1745.
- Larché, M., D.S. Robinson, and A.B. Kay. 2003. The role of T lymphocytes in the pathogenesis of asthma. *Journal of Allergy and Clinical Immunology* 111(3): 450–463.
- Romagnani, S. 2000. The role of lymphocytes in allergic disease. *Journal of Allergy and Clinical Immunology* 105(3): 399–408.
- Bradding, P., J. Roberts, K. Britten, S. Montefort, R. Djukanovic, R. Mueller, C. Heusser, P. Howarth, and S. Holgate. 1994. Interleukin-4, -5, and -6 and tumor necrosis factor- α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *American Journal of Respiratory Cell and Molecular Biology* 10(5): 471–480.
- Advenier, C., V. Lagente, and E. Boichot. 1997. The role of tachykinin receptor antagonists in the prevention of bronchial hyperresponsiveness, airway inflammation and cough. *European Respiratory Journal* 10(8): 1892–1906.
- Holgate, S., T. Casale, S. Wenzel, J. Bousquet, Y. Deniz, and C. Reisner. 2005. The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. *Journal of Allergy and Clinical Immunology* 115(3): 459–465.
- Cho, H.J., S.G. Jeong, J.E. Park, J.A. Han, H.R. Kang, D. Lee, and M.J. Song. 2013. Antiviral activity of angelicin against gammaherpesviruses. *Antiviral Research* 100(1): 75–83.
- Liu, F., G.-Q. Sun, H.-Y. Gao, R.-S. Li, L.-W. Soromou, N. Chen, Y.-H. Deng, and H.-H. Feng. 2013. Angelicin regulates LPS-induced inflammation via inhibiting MAPK/NF- κ B pathways. *Journal of Surgical Research* 185(1): 300–309.
- Wang, C.C., J.E. Lai, L.G. Chen, K.Y. Yen, and L.L. Yang. 2000. Inducible nitric oxide synthase inhibitors of Chinese herbs. Part 2: naturally occurring furanocoumarins. *Bioorganic & Medicinal Chemistry* 8(12): 2701–2707.
- Zosky, G.R., and P.D. Sly. 2007. Animal models of asthma. *Clinical and Experimental Allergy* 37(7): 973–988.
- Ackerman, K.G., H. Huang, H. Grasemann, C. Puma, J.B. Singer, A.E. Hill, E. Lander, J.H. Nadeau, G.A. Churchill, J.M. Drazen, and D.R. Beier. 2005. Interacting genetic loci cause airway hyperresponsiveness. *Physiological Genomics* 21(1): 105–111.
- Song, C., L. Luo, Z. Lei, B. Li, Z. Liang, G. Liu, D. Li, G. Zhang, B. Huang, and Z.-H. Feng. 2008. IL-17-producing alveolar macrophages mediate allergic lung inflammation related to asthma. *The Journal of Immunology* 181(9): 6117–6124.
- Kapp, A. 1993. The role of eosinophils in the pathogenesis of atopic dermatitis-eosinophil granule proteins as markers of disease activity. *Allergy* 48(1): 1–5.
- Hamid, Q., M.K. Tulic, M.C. Liu, and R. Moqbel. 2003. Inflammatory cells in asthma: mechanisms and implications for therapy. *Journal of Allergy and Clinical Immunology* 111(1): S5–S17.
- Chung, K. 1986. Role of inflammation in the hyperreactivity of the airways in asthma. *Thorax* 41(9): 657–662.
- Wakashin, H., K. Hirose, Y. Maezawa, S.-I. Kagami, A. Suto, N. Watanabe, Y. Saito, M. Hatano, T. Tokuhisa, and Y. Iwakura. 2008. IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice. *American Journal of Respiratory and Critical Care Medicine* 178(10): 1023–1032.
- Renauld, J.-C. 2001. New insights into the role of cytokines in asthma. *Journal of Clinical Pathology* 54(8): 577–589.
- Lebman, D.A., and R.L. Coffman. 1988. Interleukin 4 causes isotype switching to IgE in T cell-stimulated clonal B cell cultures. *The Journal of Experimental Medicine* 168(3): 853–862.
- Carballido, J.M., D. Schols, R. Namikawa, S. Zurawski, G. Zurawski, M.-G. Roncarolo, and J. De Vries. 1995. IL-4 induces human B cell maturation and IgE synthesis in SCID-hu mice. Inhibition of ongoing IgE production by in vivo treatment with an IL-4/IL-13 receptor antagonist. *The Journal of Immunology* 155(9): 4162–4170.
- O'Byrne, P.M., M.D. Inman, and K. Parameswaran. 2001. The trials and tribulations of IL-5, eosinophils, and allergic asthma. *Journal of Allergy and Clinical Immunology* 108(4): 503–508.
- Huffnagle, G.B., M.B. Boyd, N.E. Street, and M.F. Lipscomb. 1998. IL-5 is required for eosinophil recruitment, crystal deposition, and mononuclear cell recruitment during a pulmonary *Cryptococcus*

- neoformans infection in genetically susceptible mice (C57BL/6). *The Journal of Immunology* 160(5): 2393–2400.
22. Kuperman, D.A., X. Huang, L.L. Koth, G.H. Chang, G.M. Dolganov, Z. Zhu, J.A. Elias, D. Sheppard, and D.J. Erle. 2002. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nature Medicine* 8(8): 885–889.
23. Yang, G., A. Volk, T. Petley, E. Emmell, J. Giles-Komar, X. Shang, J. Li, A.M. Das, D. Shealy, and D.E. Griswold. 2004. Anti-IL-13 monoclonal antibody inhibits airway hyperresponsiveness, inflammation and airway remodeling. *Cytokine* 28(6): 224–232.
24. Wills-Karp, M. 2004. Interleukin-13 in asthma pathogenesis. *Immunological Reviews* 202(1): 175–190.
25. Wills-Karp, M., J. Luyimbazi, X. Xu, B. Schofield, T.Y. Neben, C.L. Karp, and D.D. Donaldson. 1998. Interleukin-13: central mediator of allergic asthma. *Science* 282(5397): 2258–2261.
26. Webb, D.C., A.N. McKenzie, A.M. Koskinen, M. Yang, J. Mattes, and P.S. Foster. 2000. Integrated signals between IL-13, IL-4, and IL-5 regulate airways hyperreactivity. *The Journal of Immunology* 165(1): 108–113.
27. Huang, C.S., A.H. Lin, T.C. Yang, K.L. Liu, H.W. Chen, and C.K. Lii. 2015. Shikonin inhibits oxidized LDL-induced monocyte adhesion by suppressing NF-kappaB activation via up-regulation of PI3K/Akt/Nrf2-dependent antioxidation in EA.hy926 endothelial cells. *Biochemical Pharmacology* 93(3): 352–361.
28. Rai, A., S. Kapoor, S. Singh, B.P. Chatterji, and D. Panda. 2015. Transcription factor NF-kappaB associates with microtubules and stimulates apoptosis in response to suppression of microtubule dynamics in MCF-7 cells. *Biochemical Pharmacology* 93(3): 277–289.
29. Zhao, Z.Z., X.F. Tang, X.H. Zhao, M.H. Zhang, W.J. Zhang, S.H. Hou, W.F. Yuan, H.F. Zhang, L.J. Shi, H. Jia, L. Liang, Z. Lai, J.F. Gao, K.Y. Zhang, L. Fu, and W. Chen. 2014. Tylvalosin exhibits anti-inflammatory property and attenuates acute lung injury in different models possibly through suppression of NF-kappa B activation. *Biochemical Pharmacology* 90(1): 73–87.
30. Bureau, F., S. Delhalle, G. Bonizzi, L. Fiévez, S. Dogné, N. Kirschvink, A. Vanderplasschen, M.-P. Merville, V. Bours, and P. Lekeux. 2000. Mechanisms of persistent NF-kB activity in the bronchi of an animal model of asthma. *The Journal of Immunology* 165(10): 5822–5830.