Baicalin attenuates inflammation in mice with OVA-induced asthma by inhibiting NF-κB and suppressing CCR7/CCL19/CCL21

JIAQI LIU[1,2*], YING WEI[1,2*], QINGLI LUO[1,2], FEI XU[1,2], ZHENGXIAO ZHAO[1,2], HONGYING ZHANG[1,2], LINWEI LU[1,2], JING SUN[1,2], FENG LIU[1,2], XIN DU[1,2], MIHUI LI[1,2], KAI WEI[1,2] and JINGCHENG DONG[1,2]

[1]Department of Integrative Medicine, Huashan Hospital, and [2]Institute of Integrated Traditional Chinese and Western Medicine, Fudan University, Shanghai 200040, P.R. China

Received December 12, 2015; Accepted September 9, 2016

DOI: 10.3892/ijmm.2016.2743

Abstract. Baicalin, extracted and purified from the Chinese medicinal plant, *Scutellaria baicalensis* Georgi (Huang qin in Chinese), exhibits potent anti-inflammatory activity against asthma. However, it remains unknown whether baicalin inhibits the activity of CC chemokine receptor 7 (CCR7) and its ligands, which are crucial for the initiation of airway inflammation. In the present study, we investigated the effects of baicalin on CCR7 and its ligands, CCL19 and CCL21, as well as on the nuclear factor-κB (NF-κB) pathway in a mouse model of asthma. A mouse model of acute asthma was established by exposing the mice to ovalbumin (OVA) (by intraperitoneal injection and inhalational challenge). Within 24 h of the final OVA challenge, lung function was detected by direct airway resistance analysis. Lung tissues were examined for pathological changes. Inflammatory cell counts in bronchoalveolar lavage fluid (BALF) were assessed. ELISA was utilized to evaluate the OVA-IgE, CCL19 and CCL21 levels in BALF. The interleukin (IL)-6 and tumor necrosis factor (TNF)-α levels in serum were also detected by ELISA. The protein expression levels of CCR7, as well as that of phosphorylated IκBα (p-IκBα) and phosphorylated p65 (p-p65) were determined by western blot analysis and RT-qPCR was used to determine the CCR7 mRNA levels. Our data demonstrated that the oral administration of baicalin significantly improved pulmonary function and attenuated inflammatory cell infiltration into the lungs. Baicalin also decreased the levels of OVA-IgE, IL-6, TNF-α and CCR7, as well as those of its ligand, CCL19; the levels of NF-κB were also markedly suppressed by baicalin. The CCR7 mRNA level was substantially decreased. Our results thus suggest that baicalin exerts an inhibitory effect on airway inflammation, and this effect may be associated with the inhibition of CCR7 and CCL19/CCL21, which may provide new mechanistic insight into the anti-inflammatory effects of baicalin.

Correspondence to: Professor Jingcheng Dong, Department of Integrative Medicine, Huashan Hospital, Fudan University, 12 Middle Urumqi Road, Shanghai 200040, P.R. China
E-mail: jcdong2004@126.com

[*]Contributed equally

Key words: asthma, baicalin, airway inflammation, CC chemokine receptor 7, CCL19/CCL21, nuclear factor-kappa B

Introduction

Bronchial asthma is a complex inflammatory airway disease mainly characterized by the presence of inflammation, airway hyperresponsiveness (AHR) and airway remodeling. While these are disease-defining characteristics, asthma has recently been recognized as a widely heterogeneous disease [Global Initiative for Asthma (GINA), 2014 update]. To date, it is estimated that 300 million individuals are affected by the disease worldwide and an effective treatment has not yet been found (1). Common allergens such as house dust, animal dander, inhalants, drugs, pollens, and air pollutants contribute to the induction of asthma. Cumulative evidence has indicated that CC chemokine receptor 7 (CCR7) and its ligands, CCL19/CCL21, are involved in these inflammatory responses (2-4); they are also associated with the recruitment and the activation of multiple cell types, such as dendritic cells (DCs), eosinophils (EOS), T cells and B cells (5-8), and also interact with inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 (9,10).

Chemokines are small (8-14 kDa) chemotactic cytokines that bind to seven transmembrane domain G protein-coupled receptors (GPCR) and are secreted by a great variety of cell types. Chemokines are important mediators involved in the migration and activation of various subsets of leukocytes (11), as well as lymphoid development and lymphocyte homing into and out of secondary and tertiary lymphoid organs.

CCR7 is a member of the GPCR family and is commonly expressed in memory T cells, B cells and mature DCs (12). CCL19 and CCL21 are the only ligands for CCR7. T cells and antigen-presenting DCs establish close physical contacts within the lymph nodes, which jointly contribute to a myriad

of adaptive immune functions, including secondary lymphoid organogenesis, and regulatory and memory T cell function (13).

It has been previously described that the CCR7 promoter includes potential binding sites for nuclear factor-κB (NF-κB) (14), which is involved in many inflammatory diseases. CCR7 is a direct target of NF-κB and the induction of CCR7 expression has been shown to be regulated by NF-κB (14), indicating that the modulation of inflammation may be beneficial to the control and treatment of asthma.

Baicalin, as a small molecule flavonoid compound (Fig. 1), which is the major active constituent of Scutellariae radix, has been extensively employed in traditional Chinese medicine (TCM) for the treatment of asthma. It has been demonstrated that baicalin exerts anti-inflammatory effects on experimental colitis (15). In a previous study of ours, we demonstrated the anti-airway remodeling effect of baicalin (16). Moreover, baicalin has been shown to rectify the Th1/Th2 and Treg/Th17 imbalance in asthmatic mice (17). However, the effects and related molecular mechanisms of action of baicalin on CCR7 and its ligands associated with airway inflammation have not been fully elucidated. The present study was designed to examine the effects and the underlying molecular mechanisms of action of baicalin on airway inflammation in a mouse model of ovalbumin (OVA)-induced asthma.

Materials and methods

Animals and reagents. Seven-week-old female BALB/c mice were purchased from SIPPR/BK Laboratory Animal Co., Ltd., (Shanghai, China), and reared under specific pathogen-free conditions following the guidelines and approval of the Animal Care and Use Committee on the Ethics of Animal Experiments of Fudan University, Shanghai, China.

Baicalin (purity ≥98%; chemical structure shown in Fig. 1) was provided by Must Biotechnology Co., Ltd. (Chengdu, China). OVA (grade V), aluminum hydroxide and methacholine (Mch) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The OVA-specific IgE ELISA kit was obtained from Shibayagi (Gunma, Japan). The CCL19 and CCL21, and the TNF-α and IL-6 ELISA kits were purchased from RayBiotech (Norcross, GA, USA). Anti-mouse CCR7 antibody was purchased from Abcam (Cambridge, MA, USA).

Mouse model of OVA-induced asthma and treatment. Asthma was initiated by an intraperitoneal injection of 0.2 ml saline containing 20 μg OVA and 2 mg aluminum hydroxide on days 0, 7, 14 and 21; from day 25 to 31, each mouse was placed into an individual chamber and challenged with 3% OVA nebulization (30 min/day) utilizing an ultrasonic nebulizer model 402AI; Yuyue, Jiangsu, China).

The mice were divided into 6 experimental groups as follows (n=12/group): i) the normal control (NC) group; ii) the asthma (A) group; iii) asthmatic mice treated with 10 mg/kg baicalin (B10) group; iv) asthmatic mice treated with 25 mg/kg baicalin (B25) group; v) asthmatic mice treated with 50 mg/kg baicalin (B50) group; and vi) asthmatic mice treated with dexamethasone (DXM) (0.085 mg/kg). The mice in the NC group were sensitized and challenged with an equivalent volume of normal saline. Baicalin and DXM were intragastrically administered from day 24 to 31 prior to the OVA challenge. The following analyses were implemented within 24 h of the final OVA challenge:

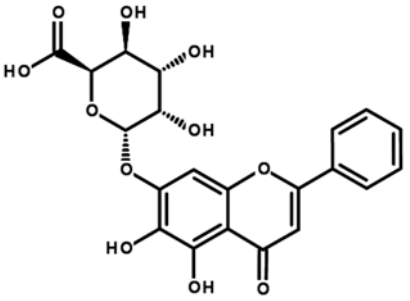

Figure 1. Molecular structure of baicalin.

Determination of lung function. AHR was detected by an invasive pulmonary facility for small animals (Buxco Electronics, Troy, NY, USA) after the final OVA challenge, as previously described by Pichavant *et al* (18). Briefly, each mouse was tracheostomized and intubated under anesthesia and the administration by nebulization to gradients of Mch (3.125, 6.25 and 12.5 mg/ml) was carried out to evaluate pulmonary resistance (R_L) and pulmonary dynamic compliance (Cdyn). Data were expressed as a percentage change from the baseline value.

Inflammatory cell classification in bronchoalveolar lavage fluid (BALF) and collection of serum. Immediately following the assessment of AHR, and while the mice were still under anesthesia, blood was collected from the left eyeball and stored at 4˚C for 2 h and then centrifuged at 5,000 x g, 4˚C for 30 min. The serum was collected, repackaged and stored at -80˚C for use in ELISA. The lung tissues of the mice were then exposed, the right lung was tied and BALF was collected by inserting a tracheal tube and lavaging the left lung 3 times with 0.3 ml aliquots of phosphate-buffered saline (PBS) and centrifuged at 800 x g, 4˚C for 10 min. The supernatants were gathered and stored at -80˚C for ELISA and the sediments were resuspended in 0.1 ml PBS for determining the cell count using a hemacytometer (Hemavet 950FS; Drew Scientific, Inc., Waterbury, CT, USA).

Lung histopathological assessment. Following the collection of BALF, the mice were euthanized and the lung tissue from the middle lobe of the right lung was detached and fixed in 4% paraformaldehyde, embedded in paraffin and thin slices (4-μm-thick) were cut from blocks and stained with hematoxylin and eosin (H&E). The histopathological changes were determined under an optical microscope (Eclipse 80i; Nikon Corporation, Tokyo, Japan) to assess the inflammatory changes in the lung tissues of the mice.

Histopathological evaluation was performed in a blinded manner on randomized sections. The severity of inflammatory cell infiltration into the lungs was assessed by a 5 point scoring system as previously described by Underwood *et al* (19).

Detection of IgE, chemokine and cytokine levels in BALF or serum by ELISA. The levels of CCL19, CCL21 and OVA-specific IgE in BALF, as well as the levels of IL-6

Table I. Primers used for PCR amplification.

Gene	Primer sequences
CCR7 (NM_001301713)	F: 5'-CGCAACTTTGAGCGGAACAA-3' R: 5'-TTCGCAGCTGCTATTGGTGA-3'
GAPDH (NM_001289726)	F: 5'-TGGCCTTCCGTGTTCCTAC-3' R: 5'-GAGTTGCTGTTGAAGTCGCA-3'

CCR7, CC chemokine receptor 7; F, forward; R, reverse.

and TNF-α in serum were detected using respective ELISA kits, following the manufacturer's instructions. The assays employed antibodies specific for each cytokine coated on 96-well plates. All reagents, samples and standards were prepared as instructed. Subsequently, 100 μl standard or sample were added to each well followed by incubation for 2.5 h at room temperature. The solution was then discarded and the sampels were washed 3-4 times with 1X Wash Solution. This was followed by the addition of 100 μl prepared biotin antibody to each well and incubation for 1 h at room temperature, and further washing with 1X Wash Solution for 3 times. Subsequently, 100 μl prepared streptavidin solution was added followed by incubation for 45 minutes at room temperature. TMB One-Step Substrate Reagent (100 μl) was then added to each well, followed by incubation for 30 min at room temperature. Finally, 50 μl Stop Solution were added to each well and the absorbance was immediately read at 450 nm.

Reverse transcription-quantitative PCR (RT-qPCR). The expression of CCR7 in the lung tissue was detected by RT-qPCR. Briefly, total RNA was extracted and reverse transcripted into first-strand cDNA. qPCR amplification was then performed according to the manufacturer's instructions using a PCR Amplifier (FTC-3000 qPCR system; Funglyn Biotech, Inc., Toronto, ON, Canada). The cycling conditions were as follows: 1 cycle at 95˚C for 3 min, 40 cycles at 95˚C for 5 sec and 60˚C for 30 sec; and 1 cycle for melting at 94˚C for 90 sec, 60˚C for 3 min and 94˚C for 10 sec. The fold change in the expression of each gene was calculated using the $2^{-\Delta\Delta Ct}$ method, with the housekeeping gene, GAPDH, as an internal control. The final data were presented as the fold change compared to the expression level in the mice in the NC group. The sequences of the primers used are listed in Table I.

Western blot analysis. Lung tissues were collected for the examination of the protein expression of CCR7, as well as that of phosphorylated (p-)IκBα and p-p65 by western blot analysis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10%) was used to isolate the total protein following extraction. The lung protein samples were electrophoretically transferred onto PVDF membranes. Subsequently, the PVDF membranes were blocked with 5% BSA for 2 h at room temperature followed by incubation with primary and secondary antibodies. The immunoblots were incubated with the following antibodies: primary antibodies overnight at 4˚C with CCR7 (1:2,000 dilution; Abcam), p-IκBα (1:1,000 dilution) and p-p65 (1:1,000 dilution) (both from Cell Signaling Technology, Inc., Danvers, MA, USA); and secondary antibodies at 37˚C for 2 h with 1:2,000 dilution. Finally, the immunoblots were visualized and analyzed using Image Lab software (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

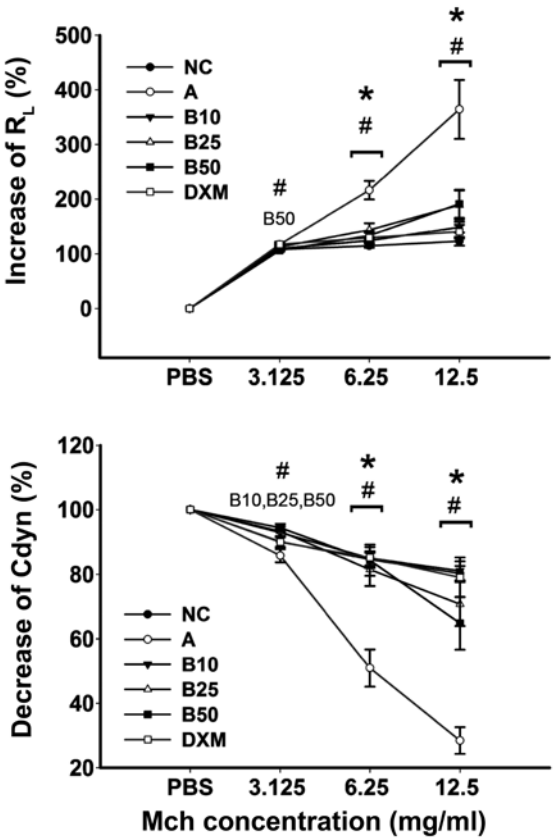

Figure 2. Baicalin reduces airway hyperresponsiveness (AHR) to elevating doses of methacholine (Mch) by decreasing airway resistance (R_L) and increasing dynamic compliance (Cdyn) in asthmatic mice. Data are expressed as a percentage change from the baseline value. *P<0.05 vs. normal control (NC) group; #P<0.05 vs. asthmaticc (A) group. B10, B25 and B50 indicates treatment with baicalin at 10, 25 and 50 mg/kg, respectively.

Statistical analysis. All analyses were undertaken using SPSS 19.0 statistical software. Data are expressed as the means ± SEM. For comparisons of multiple parameters, ANOVA with the LSD test or Dunnett's test were used to evaluate the statistical significance of the differences between groups. A P-value <0.05 was considered to indicate a statistically significant difference.

Results

Baicalin mitigates AHR in asthmatic mice. The efficacy of baicalin against OVA-induced AHR was examined by means of the airway responsiveness to aerosolized PBS or Mch. As shown in Fig. 2, only inconspicuous alterations in R_L were observed in the normal mice, while the mice with OVA-induced asthma exhibited an obvious increase in R_L and a decrease in Cdyn (Mch of 6.25 and 12.5 mg/ml) compared with the mice in the NC group (P<0.05). Treatment with all concentrations of baicalin led to an obvious increase in Cdyn

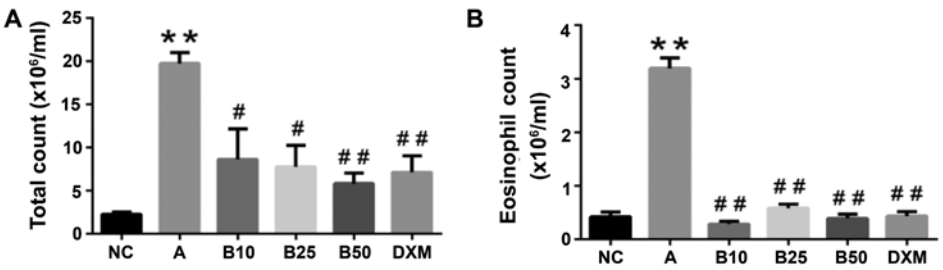

Figure 3. Baicalin inhibits the influx of inflammatory cells into the bronchoalveolar lavage fluid (BALF) of asthmatic mice. Total (A) white blood cells (WBC) and (B) eosinophils (EOS) were counted in BALF collected from mice in each group. **P<0.01 vs. normal control (NC) group; #P<0.05 vs. asthmatic (A) group; ##P<0.01 vs. A group. B10, B25 and B50 indicates treatment with baicalin at 10, 25 and 50 mg/kg, respectively.

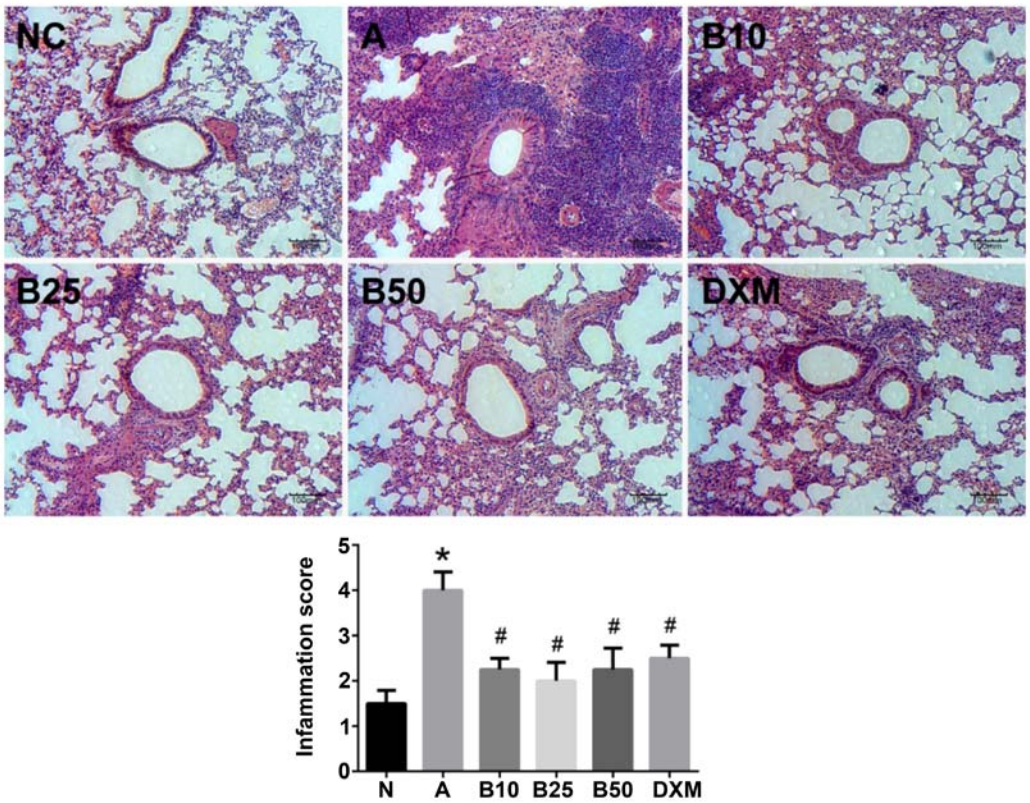

Figure 4. Baicalin attenuates the infiltration of inflammatory cells into the lungs and inhibits mucus secretion in and around the airways of asthmatic mice (magnification, x200). *P<0.05 vs. normal control (NC) group; #P<0.05 vs. asthmatic (A) group. B10, B25 and B50 indicates treatment with baicalin at 10, 25 and 50 mg/kg, respectively.

and a reduction in R_L in the asthmatic mice (P<0.05) and these effects occurred in a dose-dependent manner.

Inflammatory cell recruitment in BALF is suppressed by baicalin. As shown in by our results, the OVA-sensitized and challenged mice exhibited a large quantity of inflammatory cells infiltrating the lungs compared to the mice in the NC group (P<0.01; Fig. 3). In order to ascertain the effects of baicalin on inflammatory cell counts, we detected general white blood cells (WBCs) and EOS. The WBC and EOS counts in BALF were significantly decreased in the baicalin-treated mice. Moreover, the mice treated with 50 mg/kg baicalin (B50) exhibited a marked reduction in the WBC and EOS counts in BALF (P<0.01), and this reduction in inflammatory cell count in the B50 group was greater than that observed in the DXM-treated mice.

Histological lung inflammation caused by OVA inhalation is reduced in baicalin-treated mice. In the lung tissues from the mice exposed to OVA, a distinct infiltration of EOS and mucus secretion into the tracheobronchial mucosa and airway lumen was observed as compared with the mice in the NC group (P<0.05; Fig. 4). Conversely, the baicalin and DXM-treated mice exhibited less infiltration of EOS into the tracheobronchial mucosa and airway lumen (P<0.05).

Effect of baicalin treatment on IgE, CCL19 and CCL21 levels in OVA-challenged mice. The results of ELISA indicated

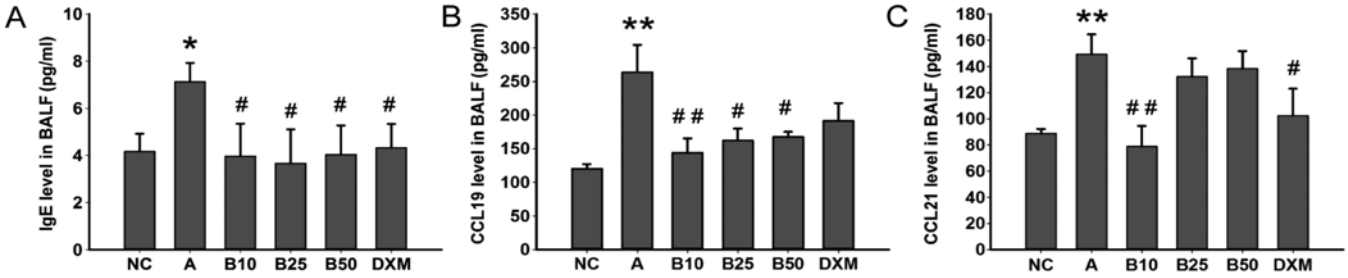

Figure 5. Baicalin reduces ovalbumin (OVA)-specific (A) IgE, (B) CCL19 and (C) CCL21 levels in bronchoalveolar lavage fluid (BALF) of asthmatic mice. OVA-specific IgE and chemokines in BALF were analyzed by ELISA. *P<0.05 vs. normal control (NC) group; **P<0.01 vs. NC group; #P<0.05 vs. asthmatic (A) group; ##P<0.01 vs. A group. B10, B25 and B50 indicates treatment with baicalin at 10, 25 and 50 mg/kg, respectively.

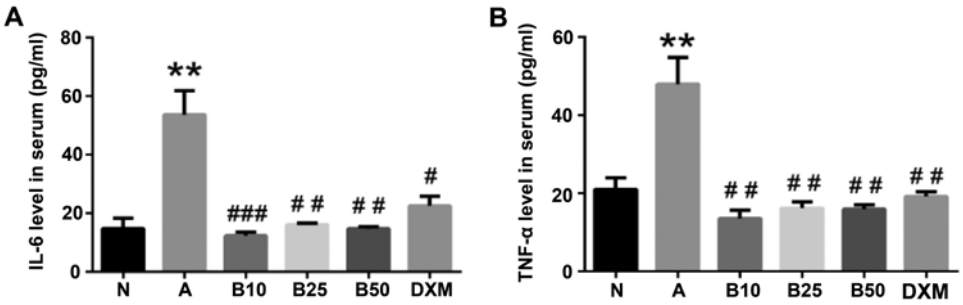

Figure 6. Baicalin inhibits the expression of inflammatory cytokines in serum of asthmatic mice. (A) IL-6 and (B) TNF-α levels were measured in serum collected from mice in each group. **P<0.01 vs. normal control (NC) group; #P<0.05 vs. asthmatic (A) group; ##P<0.01 vs. A group; ###P<0.001 vs. A group. B10, B25 and B50 indicates treatment with baicalin at 10, 25 and 50 mg/kg, respectively.

that the OVA-specific IgE level in BALF was significantly increased by the OVA challenge when compared to the mice in the NC group (P<0.05; Fig. 5A). Gavage of baicalin in each dose group showed an obvious reduction in level of OVA-specific IgE in BALF (P<0.05). Simultaneously, the mice with asthma exhibited a marked increase in the levels of CCL19 and CCL21 (P<0.01; Fig. 5B and C) in BALF. Treatment with 10 mg/kg baicalin (B10) markedly decreased the level of CCL19 and CCL21 (P<0.01), and treatment with baicalin at 25 and 50 mg/kg (B25 and B50) resulted in a marked reduction in the CCL19 level (P<0.05; Fig. 5B). However, treatment with baicalin at 25 and 50 mg/kg did not significantly inhibit the CCL21 level in BALF, whereas DXM did (Fig. 5C).

Effect of baicalin treatment on IL-6 and TNF-α levels in serum of mice with OVA-induced asthma. IL-6 and TNF-α have been shown to be implicated in many aspects of airway pathology and in evoking allergic inflammatory responses (20-22). In this study, to examine the effect of baicalin on these cytokines, the secretion of these cytokines in the serum of mice in each group were also evaluated by ELISA. As shown in Fig. 6, there were significant elevations in the expression levels of IL-6 and TNF-α in the mice with OVA-induced asthma compared with the mice in the NC group (P<0.01). The increased expression levels of these cytokines in serum were evidently suppressed by baicalin treatment (P<0.01). Enzyme immunoassays revealed that compared with the asthmatic mice, the mice treated with baicalin exhibited a marked decrease in the levels of IL-6 and TNF-α, and this effect was more prominent than that observed with DXM treatment.

Baicalin inhibits CCR7 mRNA and CCR7 protein, as well as p-IκBα and p-p65 protein expression in lung tissue. To determine whether the effects of baicalin on CCR7 and NF-κB were transcriptionally regulated, CCR7 mRNA expression was determined by RT-qPCR, and the protein expressio levels of CCR7 and p-IκBα and p-p65 were detected by western blot analysis. As shown in Fig. 7, CCR7 mRNA expression and CCR7, p-IκBα (P<0.05) and p-p65 (P<0.01) protein expression were significantly increased in the OVA-challenged mice. Notably, treatment with baicalin at 25 mg/kg (B25) had a marked inhibitory effect on the CCR7 mRNA and protein levels, as well as on the p-IκBα and p-p65 protein levels in the lung tissue of the OVA sensitized and challenged mice.

Discussion

Asthma is a highly complex airway inflammatory disorder, the incidence of which is continually increasing. Airway inflammation, AHR and mucus overproduction are the major pathological characteristics of the disease (1). Chemokine receptors with their ligands and NF-κB together with related cytokines are considered to be involved in the pathogenesis of asthma (4,23-25). In this study, the effects of baicalin on inflammation in a mouse model of OVA-induced asthmat and on the associated chemokine receptors and their ligands were investigated. Our results revealed that the OVA-exposed mice

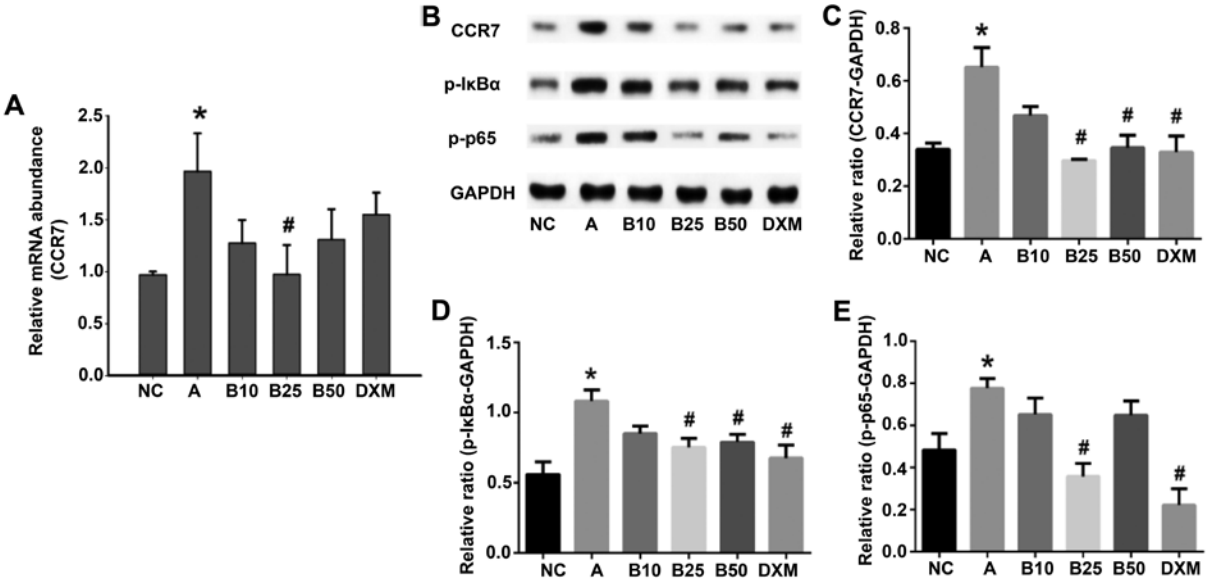

Figure 7. (A-E) Baicalin suppresses the protein expression of CC chemokine receptor 7 (CCR7) and nuclear factor (NF)-κB in the lung tissue of asthmatic mice. The levels of CCR7, as well as phosphorylated IκBα (p-IκBα) and phosphorylated p65 (p-p65) protein expression in lung tissue of mice in each group were assayed by western blot analysis. *P<0.05 vs. normal control (NC) group; #P<0.05 vs. asthmatic (A) group. B10, B25 and B50 indicates treatment with baicalin at 10, 25 and 50 mg/kg, respectively.

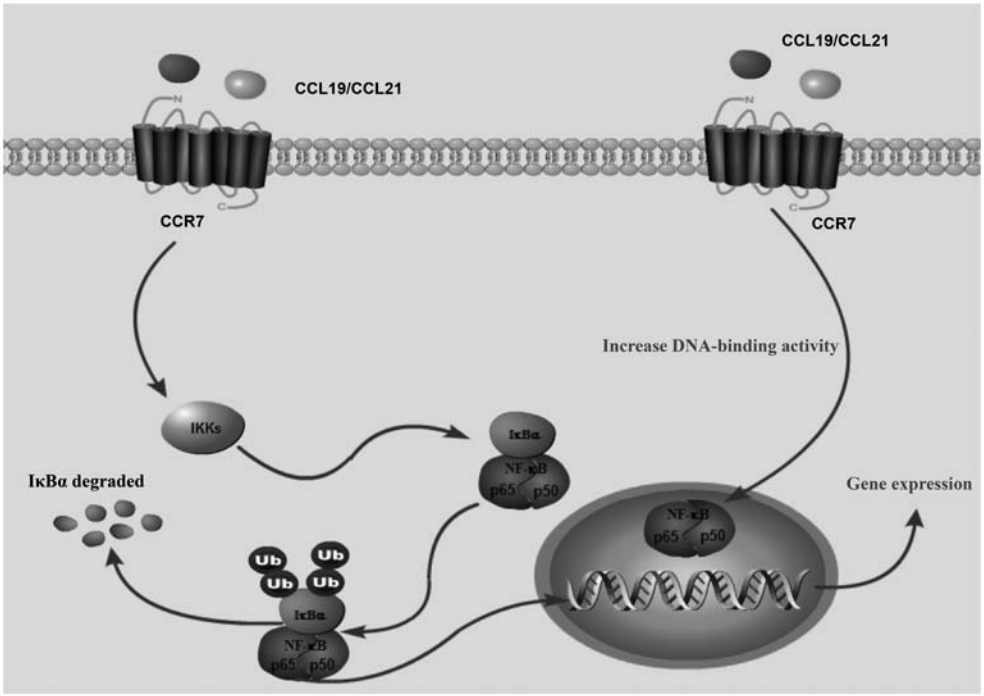

Figure 8. Possible role of CC chemokine receptor 7 (CCR7)-mediated NF-κB activation.

exhibited substantial airway inflammatory changes compared to the NC mice. Consistent with previous discoveries (26,27), in this study, AHR was markedly increased in the OVA-exposed asthmatic mice; however, treatment with baicalin led to a significant increase in Cdyn and a reduction in R_L in the asthmatic mice and these effects occurred in a dose-dependent manner, which indicated that baicalin treatment had a protective effect on pulmonary function in the asthmatic mice. Furthermore, the increased amount of inflammatory cells, as well as the OVA-specific IgE level in BALF and the secretion of inflammatory cytokines into the blood of asthmatic mice were markedly reduced by baicalin.

The homeostatic chemokines, CCL19 and CCL21, are the sole ligands for CCR7, and they have a potent chemotactic activity for antigen-presenting mDCs (28) and have been shown to be important in the homing and traffic of naive lymphocytes within lymphoid tissues (29). Studies have demonstrated that the interaction of recruited DCs and T cells at the site of regional

lymph nodes is promoted by CCL19 and CCL21 (30,31). Mice lacking both CCL21 and CCL19 gene expression exhibit a significantly reduced airway inflammatory response (4). In this study, treatment with various concentrations of baicalin exerted a marked inhibitory effect on the CCL19 level, and treatment with baicalin at 10 mg/kg significantly reduced the CCL21 level in BALF, which may support the anti-inflammatory effect of baicalin in asthmatic mice.

CCR7 is considered to be involved in the lymph node homing of naive T cells and DCs, and also plays a crucial role in regulating immune cell migration for the balance of immunity against pathogens and the maintenance of central and peripheral immunological tolerance (32,33). It has been shown that $CCR7^{-/-}$ mice exhibit a strongly impaired migration of lung-derived DCs to the bronchial lymph nodes (34). In addition, defective CCR7 expression has been shown to contribute to the reduced efficiency of the interaction between DCs and T cells, as well as to the decreased responsiveness to CCR7 ligands (35). Based on such discoveries, CCR7 has received considerable attention as a potential therapeutic target in allergies (36). In this study, the protein and mRNA expression level of CCR7 were remarkably elevated in asthmatic mice; however, CCR7 protein expression was significantly reduced by treatment with baicalin at 25 and 50 mg/kg and by DXM, and CCR7 mRNA expression was markedly decreased by treatment with baicalin at 25 mg/kg, suggesting that baicalin may be an effective medication for allergic inflammation.

Crucial to the pathogenesis of asthma, the inflammatory cytokines, IL-6 and TNF-α, which have been reported to be implicated in the airway allergic inflammatory responses, regulate many aspects in adaptive immune disorders. IL-6 and TNF-α, which can be expressed by several cell types (24,37), aggregate into the airways and lung tissues when allergy occurs. A previous study demonstrated that IL-6 levels were markedly elevated both in symptomatic and asymptomatic asthma patients (38). A recent study also found that IL-6 trans-signaling increased the expression of airway disease-related genes in airway smooth muscle (25). In addition, the anti-inflammatory effect exerted by targeting TNF-α is currently being widely investigated and studies have shown that the blockade of TNF-α contributes to the suppression of murine allergic airway inflammation (22,39). Various studies have demonstrated that the inhibition of the critical inflammatory cytokines, IL-6 and TNF-α, represent a prominent new approach for the treatment of IL-6- and TNF-α-associated inflammatory diseases (40-43). The findings of the present study demonstrated that compared with the mice in the NC group, the IL-6 and TNF-α levels in serum were significantly decreased by treatment with all concentrations of baicalin, and these effects were more prominent than those observed with DXM treatment. Our data may confirm the hypothesis that baicalin exerts an inhibitory effect on regulatory Th17 cells in asthmatic mice.

NF-κB is a master regulatory transcription factor that is crucial to the expression of inflammatory cytokines, chemokine receptors and the counterpart ligands in inflammation-related diseases (44). The inhibitor of IκB kinase (IKKs), p65/p50 complex and IκB are key cytokines in the NF-κB pathway; however, among the p65/p50 complex, only p65 has transcriptional activity (45). It has been demonstrated that the interaction between CCR7 and its ligand, CCL19, activate p-IκB and cause NF-κB to translocate to the nucleus, increasing the DNA-binding capacity of NF-κB (46). In addition, it has been demonstrated that the stimulation of DCs with CCL19 or CCL21 induces the activation of NF-κB (47). Therefore, CCR7-mediated NF-κB activation may play a key role in the pathophysiological process of asthma (Fig. 8), and the suppression of NF-κB activation and the interaction with CCR7 and its ligands are associated with reducing airway inflammation. Accordingly, we investigated whether baicalin treatment could interfere with CCR7 and the NF-κB pathway. The data from western blot analysis revealed that the expression of p-IκBα and p-p65 protein were markedly elevated in the lung tissue of OVA-exposed mice as compared to the NC mice and were markedly inhibited by treatment with baicalin at the dose of 25 mg/kg. Collectively, these data suggest the potential of baicalin to inhibit the functions of CCR7 and its ligands via NF-κB, thus attenuating inflammation, and the development and progression of asthma.

In conclusion, in the present study, we demonstrated that baicalin improves pulmonary function and attenuates airway inflammation. Our data provide novel mechanistic insight into the anti-inflammatory effects of baicalin. Importantly, the levels of IgE and CCR7, and those of its ligand, CCL19, were prominently suppressed by treatment with baicalin. Furthermore, baicalin exerts an inhibitory effect on the NF-κB pathway. These discoveries may demonstrate the potential of baicalin as an agent for the management of allergic asthma.

Acknowledgments

This study was supported by grants from the National Natural Science Foundation of China (nos. 81173390, 81403476 and 81573758) and the Development Project of Shanghai Peak Disciplines-Integrated Chinese and Western Medicine.

References

1. Olin JT and Wechsler ME: Asthma: pathogenesis and novel drugs for treatment. BMJ 349: g5517, 2014.
2. Kaur D, Saunders R, Berger P, Siddiqui S, Woodman L, Wardlaw A, Bradding P and Brightling CE: Airway smooth muscle and mast cell-derived CC chemokine ligand 19 mediate airway smooth muscle migration in asthma. Am J Respir Crit Care Med 174: 1179-1188, 2006.
3. Saunders R, Sutcliffe A, Kaur D, Siddiqui S, Hollins F, Wardlaw A, Bradding P and Brightling C: Airway smooth muscle chemokine receptor expression and function in asthma. Clin Exp Allergy 39: 1684-1692, 2009.
4. Yamashita N, Tashimo H, Matsuo Y, Ishida H, Yoshiura K, Sato K, Yamashita N, Kakiuchi T and Ohta K: Role of CCL21 and CCL19 in allergic inflammation in the ovalbumin-specific murine asthmatic model. J Allergy Clin Immunol 117: 1040-1046, 2006.
5. Sennikov SV, Falaleeva SA, Shkaruba NS, Chumasova OA, Obleukhova IA, Sizikov AE and Kurilin VV: Maturation and cytokine production potential of dendritic cells isolated from rheumatoid arthritis patients peripheral blood and induced in vitro. Hum Immunol pii: S0198-8859(16)30158-6, 2016.
6. Akuthota P, Ueki S, Estanislau J and Weller PF: Human eosinophils express functional CCR7. Am J Respir Cell Mol Biol 48: 758-764, 2013.
7. Jung YW, Kim HG, Perry CJ and Kaech SM: CCR7 expression alters memory CD8 T-cell homeostasis by regulating occupancy in IL-7- and IL-15-dependent niches. Proc Natl Acad Sci USA 113: 8278-8283, 2016.
8. Moschovakis GL, Bubke A, Dittrich-Breiholz O, Braun A, Prinz I, Kremmer E and Förster R: Deficient CCR7 signaling promotes TH2 polarization and B-cell activation in vivo. Eur J Immunol 42: 48-57, 2012.

9. Boislève F, Kerdine-Romer S, Rougier-Larzat N and Pallardy M: Nickel and DNCB induce CCR7 expression on human dendritic cells through different signalling pathways: role of TNF-alpha and MAPK. J Invest Dermatol 123: 494-502, 2004.
10. Pahne-Zeppenfeld J, Schröer N, Walch-Rückheim B, Oldak M, Gorter A, Hegde S and Smola S: Cervical cancer cell-derived interleukin-6 impairs CCR7-dependent migration of MMP-9-expressing dendritic cells. Int J Cancer 134: 2061-2073, 2014.
11. Johnston B and Butcher EC: Chemokines in rapid leukocyte adhesion triggering and migration. Semin Immunol 14: 83-92, 2002.
12. Ben-Baruch A: Organ selectivity in metastasis: regulation by chemokines and their receptors. Clin Exp Metastasis 25: 345-356, 2008.
13. Comerford I, Harata-Lee Y, Bunting MD, Gregor C, Kara EE and McColl SR: A myriad of functions and complex regulation of the CCR7/CCL19/CCL21 chemokine axis in the adaptive immune system. Cytokine Growth Factor Rev 24: 269-283, 2013.
14. Höpken UE, Foss HD, Meyer D, Hinz M, Leder K, Stein H and Lipp M: Up-regulation of the chemokine receptor CCR7 in classical but not in lymphocyte-predominant Hodgkin disease correlates with distinct dissemination of neoplastic cells in lymphoid organs. Blood 99: 1109-1116, 2002.
15. Cui L, Feng L, Zhang ZH and Jia XB: The anti-inflammation effect of baicalin on experimental colitis through inhibiting TLR4/NF-κB pathway activation. Int Immunopharmacol 23: 294-303, 2014.
16. Sun J, Li L, Wu J, Liu B, Gong W, Lv Y, Luo Q, Duan X and Dong J: Effects of baicalin on airway remodeling in asthmatic mice. Planta Med 79: 199-206, 2013.
17. Ma C, Ma Z, Fu Q and Ma S: Anti-asthmatic effects of baicalin in a mouse model of allergic asthma. Phytother Res 28: 231-237, 2014.
18. Pichavant M, Goya S, Hamelmann E, Gelfand EW and Umetsu DT: Animal models of airway sensitization. Curr Protoc Immunol Chapter 15: Unit 15.18, 2007.
19. Underwood S, Foster M, Raeburn D, Bottoms S and Karlsson JA: Time-course of antigen-induced airway inflammation in the guinea-pig and its relationship to airway hyperresponsiveness. Eur Respir J 8: 2104-13, 1995.
20. Chu DK, Al-Garawi A, Llop-Guevara A, Pillai RA, Radford K, Shen P, Walker TD, Goncharova S, Calhoun WJ, Nair P and Jordana M: Therapeutic potential of anti-IL-6 therapies for granulocytic airway inflammation in asthma. Allergy Asthma Clin Immunol 11: 14, 2015.
21. Rose-John S, Scheller J, Elson G and Jones SA: Interleukin-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer. J Leukoc Biol 80: 227-236, 2006.
22. Brightling C, Berry M and Amrani Y: Targeting TNF-α: a novel therapeutic approach for asthma. J Allergy Clin Immunol 121: 5-10, quiz 11-12, 2008.
23. Gagliardo R, Chanez P, Profita M, Bonanno A, Albano GD, Montalbano AM, Pompeo F, Gagliardo C, Merendino AM and Gjomarkaj M: IkappaB kinase-driven nuclear factor-kappaB activation in patients with asthma and chronic obstructive pulmonary disease. J Allergy Clin Immunol 128: 635-645, 2011.
24. Berry M, Brightling C, Pavord I and Wardlaw A: TNF-α in asthma. Curr Opin Pharmacol 7: 279-282, 2007.
25. Robinson MB, Deshpande DA, Chou J, Cui W, Smith S, Langefeld C, Hastie AT, Bleecker ER and Hawkins GA: IL-6 trans-signaling increases expression of airways disease genes in airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 309: L129-L138, 2015.
26. Sun J, Wu J, Xu C, Luo Q, Li B and Dong J: Paeoniflorin attenuates allergic inflammation in asthmatic mice. Int Immunopharmacol 24: 88-94, 2015.
27. Wei Y, Luo QL, Sun J, Chen MX, Liu F and Dong JC: Bu-Shen-Yi-Qi formulae suppress chronic airway inflammation and regulate Th17/Treg imbalance in the murine ovalbumin asthma model. J Ethnopharmacol 164: 368-377, 2015.
28. Le Y, Zhou Y, Iribarren P and Wang J: Chemokines and chemokine receptors: their manifold roles in homeostasis and disease. Cell Mol Immunol 1: 95-104, 2004.
29. Ebert LM, Schaerli P and Moser B: Chemokine-mediated control of T cell traffic in lymphoid and peripheral tissues. Mol Immunol 42: 799-809, 2005.
30. Lambrecht BN: Allergen uptake and presentation by dendritic cells. Curr Opin Allergy Clin Immunol 1: 51-59, 2001.
31. Choi B, Lim HC, Lee ES, Anower AKMM and Sohn S: CCL21 attenuates HSV-induced inflammation through up-regulation of $CD8^+$ memory cells. Immunobiology 218: 579-590, 2013.
32. Förster R, Davalos-Misslitz AC and Rot A: CCR7 and its ligands: balancing immunity and tolerance. Nat Rev Immunol 8: 362-371, 2008.
33. Worbs T and Förster R: A key role for CCR7 in establishing central and peripheral tolerance. Trends Immunol 28: 274-280, 2007.
34. Hintzen G, Ohl L, del Rio ML, Rodriguez-Barbosa JI, Pabst O, Kocks JR, Krege J, Hardtke S and Förster R: Induction of tolerance to innocuous inhaled antigen relies on a CCR7-dependent dendritic cell-mediated antigen transport to the bronchial lymph node. J Immunol 177: 7346-7354, 2006.
35. Ato M, Stäger S, Engwerda CR and Kaye PM: Defective CCR7 expression on dendritic cells contributes to the development of visceral leishmaniasis. Nat Immunol 3: 1185-1191, 2002.
36. Saban DR: The chemokine receptor CCR7 expressed by dendritic cells: a key player in corneal and ocular surface inflammation. Ocul Surf 12: 87-99, 2014.
37. Schmidt-Weber CB, Akdis M and Akdis CA: TH17 cells in the big picture of immunology. J Allergy Clin Immunol 120: 247-54, 2007.
38. Yokoyama A, Kohno N, Fujino S, Hamada H, Inoue Y, Fujioka S, Ishida S and Hiwada K: Circulating interleukin-6 levels in patients with bronchial asthma. Am J Respir Crit Care Med 151: 1354-1358, 1995.
39. Hutchison S, Choo-Kang BSW, Bundick RV, Leishman AJ, Brewer JM, McInnes IB and Garside P: Tumour necrosis factor-α blockade suppresses murine allergic airways inflammation. Clin Exp Immunol 151: 114-122, 2008.
40. Hunter CA and Jones SA: IL-6 as a keystone cytokine in health and disease. Nat Immunol 16: 448-457, 2015.
41. Nishimoto N and Kishimoto T: Inhibition of IL-6 for the treatment of inflammatory diseases. Curr Opin Pharmacol 4: 386-391, 2004.
42. Berry MA, Hargadon B, Shelley M, Parker D, Shaw DE, Green RH, Bradding P, Brightling CE, Wardlaw AJ and Pavord ID: Evidence of a role of tumor necrosis factor alpha in refractory asthma. N Engl J Med 354: 697-708, 2006.
43. Howarth PH, Babu KS, Arshad HS, Lau L, Buckley M, McConnell W, Beckett P, Al Ali M, Chauhan A, Wilson SJ, *et al*: Tumour necrosis factor (TNF alpha) as a novel therapeutic target in symptomatic corticosteroid dependent asthma. Thorax 60: 1012-1018, 2005.
44. Kuwabara T, Tanaka Y, Ishikawa F, Kondo M, Sekiya H and Kakiuchi T: CCR7 ligands up-regulate IL-23 through PI3-kinase and NF-kappa B pathway in dendritic cells. J Leukoc Biol 92: 309-318, 2012.
45. Zhang H and Sun SC: NF-κB in inflammation and renal diseases. Cell Biosci 5: 63, 2015.
46. Liu FY, Zhao ZJ, Li P, Ding X, Guo N, Yang LL, Zong ZH and Sun CF: NF-κB participates in chemokine receptor 7-mediated cell survival in metastatic squamous cell carcinoma of the head and neck. Oncol Rep 25: 383-391, 2011.
47. Sánchez-Sánchez N, Riol-Blanco L, de la Rosa G, Puig-Kröger A, García-Bordas J, Martín D, Longo N, Cuadrado A, Cabañas C, Corbí AL, *et al*: Chemokine receptor CCR7 induces intracellular signaling that inhibits apoptosis of mature dendritic cells. Blood 104: 619-625, 2004.

Reproduced with permission of copyright owner.
Further reproduction prohibited without permission.