

FcγR/ROS/CK2α Is the Key Inducer of NF-κB Activation in a Murine Model of Asthma

June-Mo Kim^a Hae-Kyoung Kim^a Yoo-Na Im^a Yun-Soo Bae^b
Suhn-Young Im^c Hern-Ku Lee^a

^aDepartment of Immunology, Chonbuk National University Medical School, Jeonju, ^bDepartment of Life Science and GT5 Program, Ewha Womans University, Seoul, and ^cDepartment of Biological Sciences, College of Natural Sciences, Chonnam National University, Gwangju, Republic of Korea

Keywords

Asthma · Nuclear factor-κB · CK2α · Reactive oxygen species · Fcγ receptor

Abstract

Background: The transcription factor nuclear factor (NF)-κB plays a pivotal role in the development of allergic airway inflammation. However, the mechanism of NF-κB activation in asthma remains to be elucidated. **Methods:** CK2α activation was assessed by CK2α phosphorylation and protein expression. Airway levels of histamine and cytokines were determined by ELISA. We used 2 (active and passive) forms of allergic pulmonary inflammation models. In the active form, the animals were immunized with ovalbumin (OVA) intraperitoneally, followed by an airway challenge with OVA. In the passive form, the animals were passively sensitized by intratracheal instillation with either anti-OVA IgE or anti-

OVA IgG, followed by an airway challenge with OVA. The role of NADPH oxidase (NOX) in CK2α activation was assessed using NOX2^{-/-} and NOX4^{-/-} mice because NOX2 and NOX4 contribute to many inflammatory diseases. **Results:** The second airway challenge increased CK2α phosphorylation and protein expression in airway epithelial cells as well as nuclear translocation of the p50 and p65 subunits of NF-κB, all of which were inhibited by the CK2α inhibitor 4,5,6,7-tetrabromobenzotriazole and the antioxidant N-acetyl-L-cysteine. CK2α phosphorylation and protein expression were significantly impaired in NOX2^{-/-}, but not in NOX4^{-/-}, mice. Induction of passive sensitization using anti-OVA IgE activated neither CK2α nor NF-κB. In contrast, induction of passive sensitization using anti-OVA IgG activated both CK2α and NF-κB. **Conclusions:** These data suggest that Fcγ receptor/reactive oxygen species/CK2α is a key inducer of NF-κB activation in airway epithelial cells in a murine model of asthma.

© 2018 S. Karger AG, Basel

J.-M.K., H.-K.K., and Y.-N.I. contributed equally to this work.
Edited by: D. Dombrowicz, Lille.

Correspondence to: Prof. Hern-Ku Lee
Department of Immunology
Chonbuk National University Medical School
Jeonju, Jeollabuk-do 561-756 (Republic of Korea)
E-Mail leeh-k@jbnu.ac.kr

Co-corresponding author: Prof. Suhn-Young Im
Department of Biological Sciences
College of Natural Sciences, Chonnam National University
Gwangju 500-757 (Republic of Korea)
E-Mail syim@chonnam.ac.kr

Introduction

Airway inflammation is a cardinal feature of bronchial asthma leading to airway hyperresponsiveness, airway obstruction, mucus production, and airway wall remodeling. The inflammation is induced by the release of potent chemical mediators from inflammatory cells, such as mast cells, neutrophils, lymphocytes, and eosinophils, which are recruited into the airways. The transcription factor nuclear factor (NF)- κ B, induces various genes involved in inflammatory responses [1, 2]. Many of the inflammatory proteins that are expressed in asthmatic airways are regulated, at least in part, by NF- κ B. These include cytokines (e.g., interleukin [IL]-1 β , IL-4, IL-5, IL-9, IL-15, and tumor necrosis factor [TNF]- α), chemokines (e.g., RANTES, monocyte chemoattractant protein-3, and eotaxin), and adhesion molecules (e.g., ICAM-1 and VCAM-1). In fact, it has been shown that NF- κ B plays a key role in the development of all cardinal features of asthma, including airway hyperresponsiveness, airway obstruction, and airway eosinophilic inflammation [3–7]. Thus, understanding the mechanisms of NF- κ B activation in asthma might provide an interesting approach to improve asthma management. However, despite its importance, these mechanisms remain to be established.

The protein kinase CK2 α (formerly casein kinase 2 or II) is a highly conserved and ubiquitous protein serine/threonine (Ser/Thr) kinase which is localized to the cell nucleus and cytoplasm. It consists of 2 catalytic subunits (42-kDa α and 38-kDa α') and a regulatory subunit (28-kDa β), producing holoenzyme structures, linking the catalytic subunits through the β subunit forming $\alpha 2\beta 2$, $\alpha\alpha'\beta 2$, or $\alpha'2\beta 2$ heterotetramers [8]. CK2 α impacts over 300 potential substrates in the cell and a wide range of pathways pertaining to cell growth, proliferation, and apoptosis [9]. CK2 α plays a critical role in the development of various types of cancers, and it has been found to be dysregulated in all of the cancers examined [10, 11].

CK2 α also regulates inflammation by phosphorylating, thereby modulating the actions of several transcription factors such as NF- κ B, signal transducer and activator of transcription 1, cyclic adenosine monophosphate (cAMP) response element-binding protein, cAMP response element modulator protein, PU.1, specificity protein-1, CCAAT-enhancer-binding proteins, and steroid hormone receptors [12, 13]. Among them, NF- κ B has received considerable attention pertaining to a link with CK2 α signaling. CK2 α has been shown to

phosphorylate the inhibitor of κ B (I κ B α) at a number of sites, triggering degradation of the protein, resulting in the activation of NF- κ B [14, 15]. In addition to I κ B, CK2 α has been shown to phosphorylate the NF- κ B family member RelA/p65 following TNF- α stimulation, which results in phosphorylation of Ser 539, thereby enhancing the DNA transactivation potential of NF- κ B [16]. This suggests the possibility that CK2 α participates in airway inflammation via the activation of NF- κ B. However, the role of CK2 α in asthma is still poorly understood.

In the present study, we focused on elucidating the role of CK2 α in NF- κ B activation in a murine model of allergic asthma. We found that CK2 α plays a role in NF- κ B activation in a reactive oxygen species (ROS)-dependent manner, and that ligation of the Fc γ receptor (Fc γ R), but not the Fc ϵ receptor (Fc ϵ R), was involved in the production of ROS.

Methods

Animals

Specific pathogen-free female BALB/c mice were purchased from Samtako Inc. (Osan, Korea). Balb/c mice purchased from Orient Bio (Gapyeong, Gyeonggi, Korea) were housed in a laminar flow cabinet and provided standard laboratory chow ad libitum. NOX2 knockout (NOX2^{-/-}) mice (C57BL/6 background) and their littermates were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). NOX4^{-/-} (C57BL/6 background) mice were generated as described previously [17]. The mice were 7–8 weeks old at the start of each experiment. All of the mice used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the Chonbuk National University Medical School.

Reagents and Antibodies

Ovalbumin (OVA) (grade V) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The CK2 inhibitor 4,5,6,7-tetrabromobenzotriazole (TBBt), purchased from Calbiochem Merck (Darmstadt, Germany), was administered via intraperitoneal (i.p.) injection (12.5 mg/kg) 1 h before the second airway challenge. The antioxidant, N-acetyl-L-cysteine (NAC) from Sigma-Aldrich was administered i.p. (20 mg/kg) 1 h before the second airway challenge. Anti-CK2 α , anti-p65, and anti-phospho-p65 (Ser 539) were from Cell Signaling Technology (Beverly, MA, USA), and anti-p50 and anti-GAPDH were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-phospho-CK2 α (Thr306 and Ser362), anti-OVA IgE, and anti-OVA IgG were purchased from Chondrex (Redmond, WA, USA) and Sigma-Aldrich, respectively.

Active Immunization and Challenge

Active immunization and challenge were performed as described previously [18], with some modifications. Mice were immunized i.p. with 20 μ g OVA plus 1.0 mg aluminum hydroxide

adjuvant on day 0 and OVA alone on day 10. The immunized mice were exposed to aerosolized OVA on days 17 and 24. Aerosolization of OVA was performed using a chamber adapted for mice. Animals were exposed to 1% OVA using an ultrasonic nebulizer (NE-U12; Omron, Tokyo, Japan; output 0.8 mL/min) for 20 min. Control animals received the same immunization and the first airway challenge of OVA but were exposed to aerosolized saline instead of OVA during the second airway challenge.

Passive Immunization and Challenge

Mice were passively sensitized by intratracheal (i.t.) instillation with 1 µg of anti-OVA IgE or anti-OVA IgG. The animals were exposed to 1% OVA for 20 min 24 h and 1 h after instillation of IgE and IgG, respectively.

Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) was performed as described previously [18]. Mice were anaesthetized and the trachea cannulated while the thorax was gently massaged. The lungs were lavaged with 800 µL saline. Equal volumes of BAL fluid samples were centrifuged and the supernatant was stored at -70°C until assay for cytokines. The pellet was resuspended in saline and differential cell counts were made by staining cytopsin preparations of BAL cells with Diff-Quik (International Reagents Corp., Kobe, Japan). The different cell types were enumerated based on their morphology and staining profile.

Immunohistochemistry

The lungs were fixed in 4% neutral buffered formalin (Sigma Chemical, St. Louis, MO, USA) overnight at room temperature, paraffin embedded, and sectioned at 4 µm. Immunoperoxidase staining was performed at room temperature using a Histostain-Plus Kit (ZYMED Lab. Inc., San Francisco, CA, USA), as described previously [19].

ELISA

The levels of TNF-α, keratinocyte-derived chemokine (KC), macrophage inflammatory protein (MIP)-2, and histamine in BAL fluids were determined via ELISA. The lower limits of detection for the cytokines and histamine were as follows: TNF-α: >5.1 pg/mL (R&D Systems), KC: >2 pg/mL (R&D Systems), MIP-2: >1.5 pg/mL (R&D Systems), and histamine: >0.3 ng (ALPCO, Windham, NH, USA).

Immunoblotting Analysis

The mice were sacrificed by cervical dislocation. Immediately thereafter, the lungs were removed washed with cold saline and then dried with blotting paper. Equal amounts of cell or tissue extracts were separated on a 10% SDS polyacrylamide gel under the reducing condition and transferred onto PVDF membranes (GE Healthcare, Fairfield, CT, USA). The membranes were blocked for 1 h at room temperature using 5% skimmed milk in TBS-0.1% Tween 20 and then incubated overnight at 4°C for with primary antibodies (Abs). The blots were washed for 25 min in 5 changes of TBS (0.1% Tween 20) solution, followed by incubation for 1.5 h with horseradish peroxidase-conjugated goat anti-rabbit IgG and horseradish peroxidase-conjugated IgG Abs (Enzo Life Sciences Inc., Farmingdale, NY, USA). After washing 5 times for 25 min, they were developed using an ECL Advance Western Blotting Detection Kit (GE Healthcare).

Statistical Analysis

All data are expressed as means ± SD. Statistical comparisons were performed using one-way ANOVA followed by the Fisher exact test. Significant differences between groups were determined using an unpaired Student's *t* test. *p* < 0.05 was considered statistically significant.

Results

Airway Allergen Challenge Increases CK2α Phosphorylation and Protein Expression

CK2α phosphorylation was increased in the lungs upon a second airway challenge, reaching a peak at 10–30 min (Fig. 1a). We examined CK2α protein expression via Western blotting and immunohistochemistry. Western blot analysis revealed that airway challenge increased CK2α expression in the lungs, reaching a maximum at 1–2 h (Fig. 1b). Immunohistochemistry demonstrated CK2α expression with kinetics similar to those observed in Western blotting (Fig. 1c). CK2α was strongly expressed in airway epithelial cells as well as in pneumocytes and endothelial cells. CK2α was stained in the cytoplasm and the nucleus at relatively early time points (0–30 min), but it was stained mainly within the nucleus at 2–4 h. The increases in CK2α phosphorylation and protein expression were blocked by pretreatment with the CKα inhibitor TBBt (Fig. 1a–c).

CK2α Is Involved in NF-κB Activation in Asthmatic Lungs

NF-κB activation was determined by translocation of the p50 and p65 subunits of NF-κB to the nucleus (Fig. 2a) and by the known CK2α-dependent phosphorylation sites of p65 (Ser 539) (Fig. 2b). All of the parameters were increased, reaching a maximum at 30 min to 2 h after the challenge, and they were inhibited by pretreatment with TBBt (Fig. 2a, b). We assessed the BAL levels of the NF-κB-dependent proinflammatory cytokines TNF-α, MIP-1, and KC, all of which reached a maximum within 2 h postchallenge [20]. TBBt suppressed the BAL levels of all of the cytokines examined (Fig. 2c–e). A second OVA challenge increased the numbers of total cells, neutrophils, eosinophils, and lymphocytes in BAL fluids compared to the numbers in saline-challenged mice, and the increase in the numbers of these cells was significantly reduced by the administration of TBBt (Fig. 2f). TBBt reduced the neutrophil number at the same concentration (12.5 mg/kg) which exhibited CK2α inhibition as seen in Fig. 1. However, a 2-fold higher concentration of TBBt was required to reduce the number of eosinophils and lymphocytes in BAL fluids.

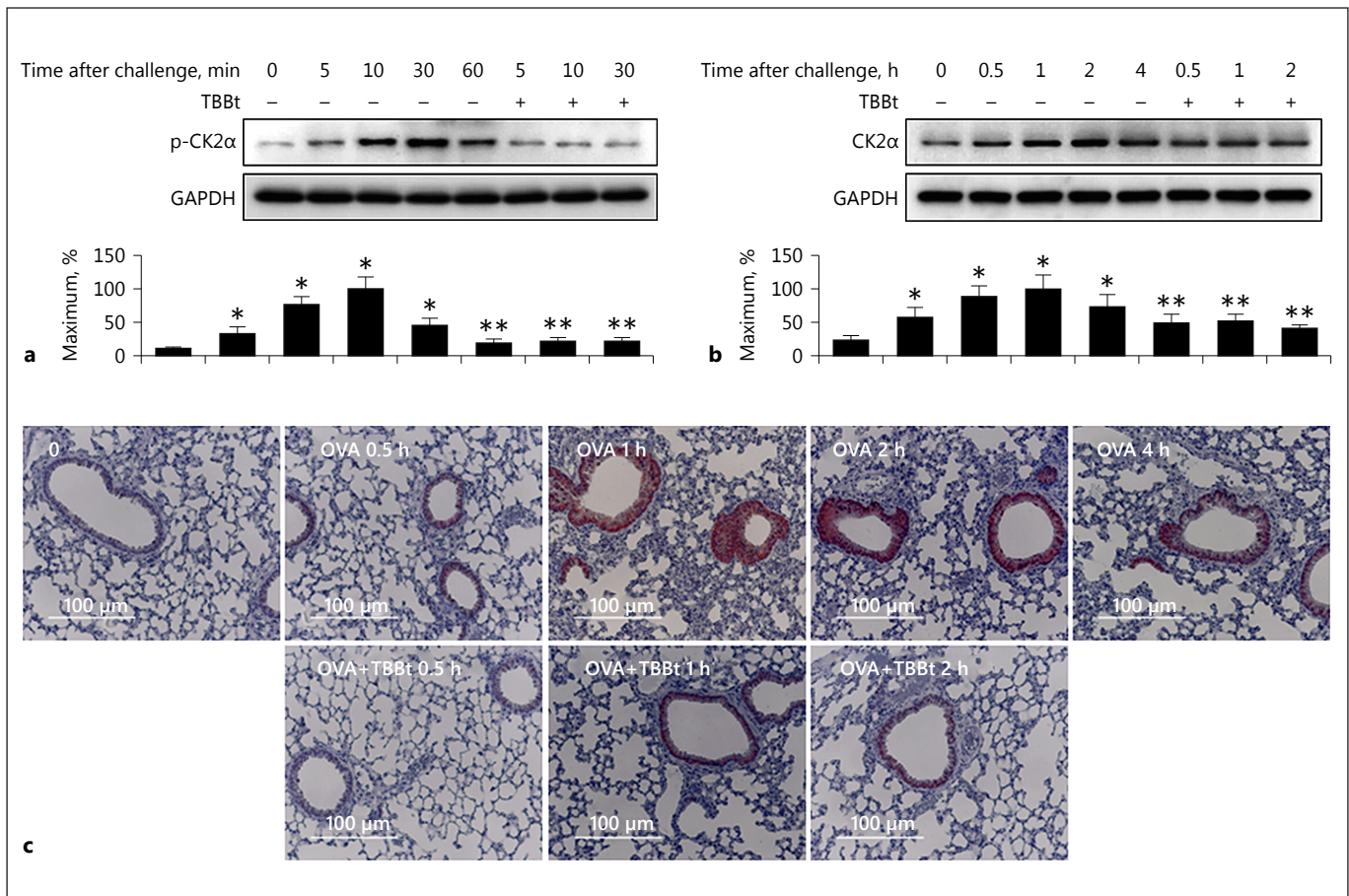


Fig. 1. Airway allergen challenge increases CK2α phosphorylation and protein expression. The lungs were removed at the time indicated from the beginning of the second airway challenge, and the following assays were performed: Western blotting of CK2α phosphorylation (**a**), Western blotting of CK2α protein expression (**b**),

and immunohistochemistry for CK2α protein expression (**c**). Data are representative of 3–5 independent experiments with 3–5 mice per time point per experiment. * $p < 0.05$ vs. control (“0” point); ** $p < 0.05$ vs. OVA group. OVA, ovalbumin.

Mechanism of CK2α Activation in Allergic Asthma: The Role of ROS

The upstream pathways leading to CK2α activation in asthma are currently unknown. Hence, we investigated the role of ROS in CK2α activation based on previous reports showing that ROS acts as a CK2α inducer via the activation of p38 mitogen-activated protein kinase [19]. Pretreatment of OVA-sensitized/-challenged mice with NAC resulted in the inhibition of CK2α phosphorylation (Fig. 3a) and protein expression (Fig. 3a, b). NAC pretreatment reduced the airway recruitment of neutrophils and eosinophils, but a 3-fold higher concentration of NAC was required to reduce the number of eosinophils (Fig. 3c). These data suggest that ROS plays an important role in CK2α activation. To further explore the role of

ROS, we examined the effect of NADPH oxidase (NOX) on CK2α phosphorylation and protein expression using NOX2^{-/-} and NOX4^{-/-} mice. CK2α phosphorylation and protein expression were significantly impaired in NOX2^{-/-} (Fig. 3d, e) but not in NOX4^{-/-} mice (Fig. 3g, h). When we compared the number of inflammatory cells in BAL fluids between NOX2^{-/-} and NOX4^{-/-} mice, a much lower cellularity was observed in NOX2^{-/-} mice (Fig. 3f, i). Our data suggest the importance of NOX2 in CK2α phosphorylation and protein expression.

Role of Allergen-Specific IgE and IgG Abs

As an upstream signaling event leading to ROS production, we examined the role of Fc receptor ligation based on the facts that: (i) the induction of allergen-in-

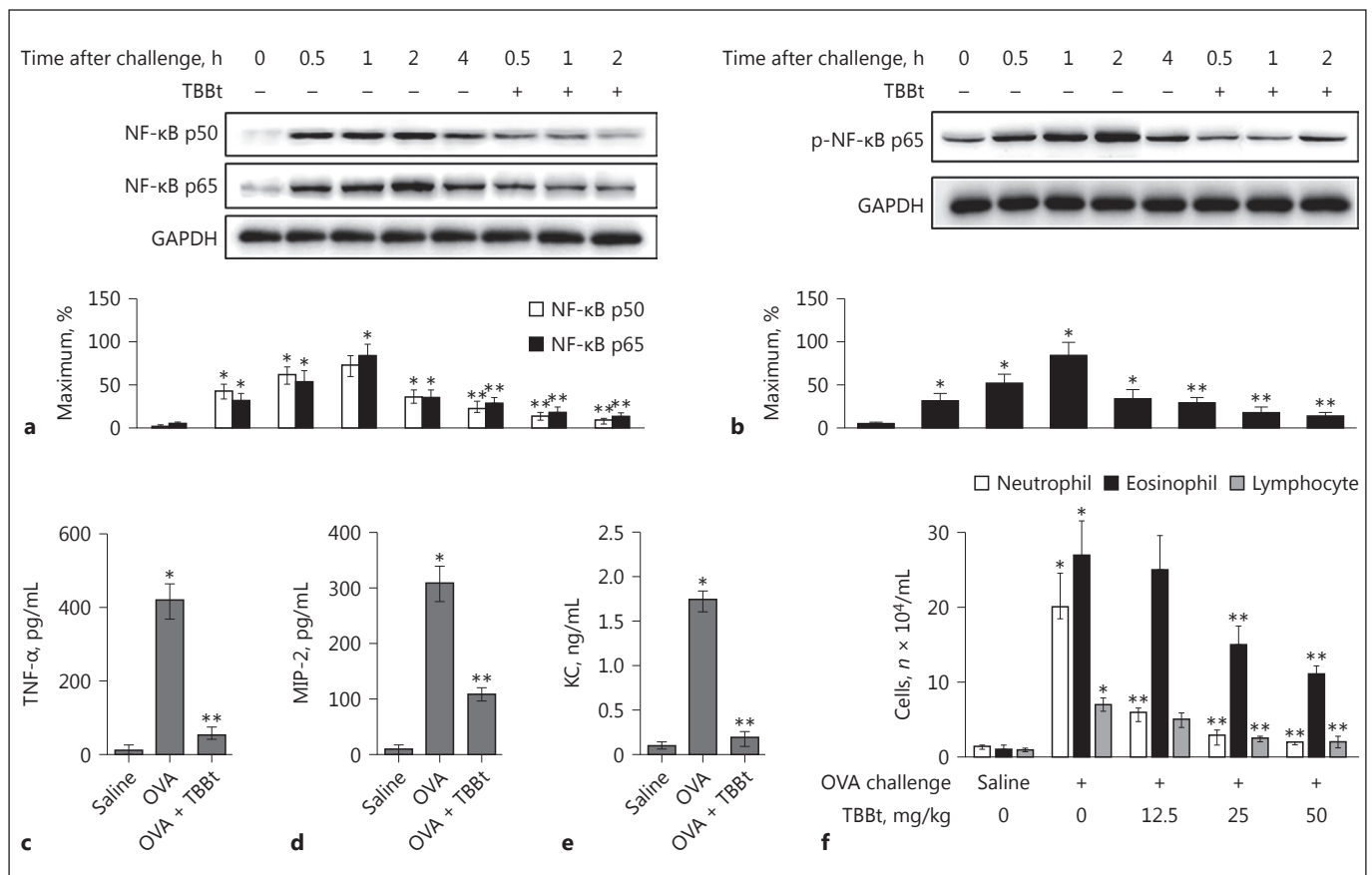


Fig. 2. CK2 α inhibitor inhibits airway challenge-induced nuclear factor (NF)- κ B activation. The CK2 inhibitor TBBt (12.5 mg/kg) was intraperitoneally administered 1 h before the second airway challenge. The lungs were removed at the time indicated from the beginning of the second airway challenge, and the following assays were performed: Western blotting of the p50 and p65 subunits of NF- κ B (**a**), Western blotting of phosphorylation of p65 (Ser 539) (**b**), and measurement of BAL levels of TNF- α (**c**), keratinocyte-derived chemokine (KC) (**d**), and macrophage inflammatory protein (MIP)-2 (**e**) at 2 h after challenge. **f** Measurement of the num-

bers of neutrophils at 8 h after challenge and eosinophils and lymphocytes at 36 h after challenge in BAL fluids. **a, b** Data are representative of 3–5 independent experiments with 3–5 mice per time point per experiment. **c–e** Data are presented as the mean $\pm$ SEM of 3 independent experiments with 3–5 mice per group per experiment. **a, b** * $p < 0.05$ vs. control (“0” point). ** $p < 0.05$ vs. ovalbumin (OVA)-challenged group. **c–e** * $p < 0.01$ vs. control group and ** $p < 0.05$ vs. OVA-challenged group. **f** * $p < 0.05$ vs. saline group and ** $p < 0.05$ vs. OVA-challenged group.

duced asthma should be initiated from the binding of allergen and allergen-specific IgE and IgG and (ii) antigen ligation of Fc γ R1 and Fc ϵ R can lead to the generation of ROS [21–23]. For this purpose, we developed a murine model of passive allergic pulmonary inflammation in which the mice were sensitized by the passive transfer of anti-OVA IgE (1 μ g) or anti-OVA IgG (1 μ g) into the airway through i.t. instillation. This was followed by an airway challenge with 1% OVA for 20 min 24 h and 1 h after instillation of IgE and IgG, respectively. The optimal conditions were determined in a preliminary experiment. The suitability of this model to induce an asth-

matic reaction was first evaluated by measuring: (i) BAL levels of histamine as an indicator of mast cell degranulation, (ii) NF- κ B activation in lung tissues, and (iii) BAL levels of the NF- κ B-dependent proinflammatory cytokines (TNF- α , MIP-2, and KC). Instillation of anti-OVA IgE followed by an airway OVA challenge (IgE sensitization/OVA challenge) resulted in increased levels of histamine (Fig. 4a) and TNF- α (Fig. 4c) in BAL fluids; however, NF- κ B activation did not occur in the mice (Fig. 4b). In contrast, IgG sensitization/OVA challenge released less histamine (Fig. 4a) but induced the activation of NF- κ B (Fig. 4b) and all 3 proinflammatory cyto-

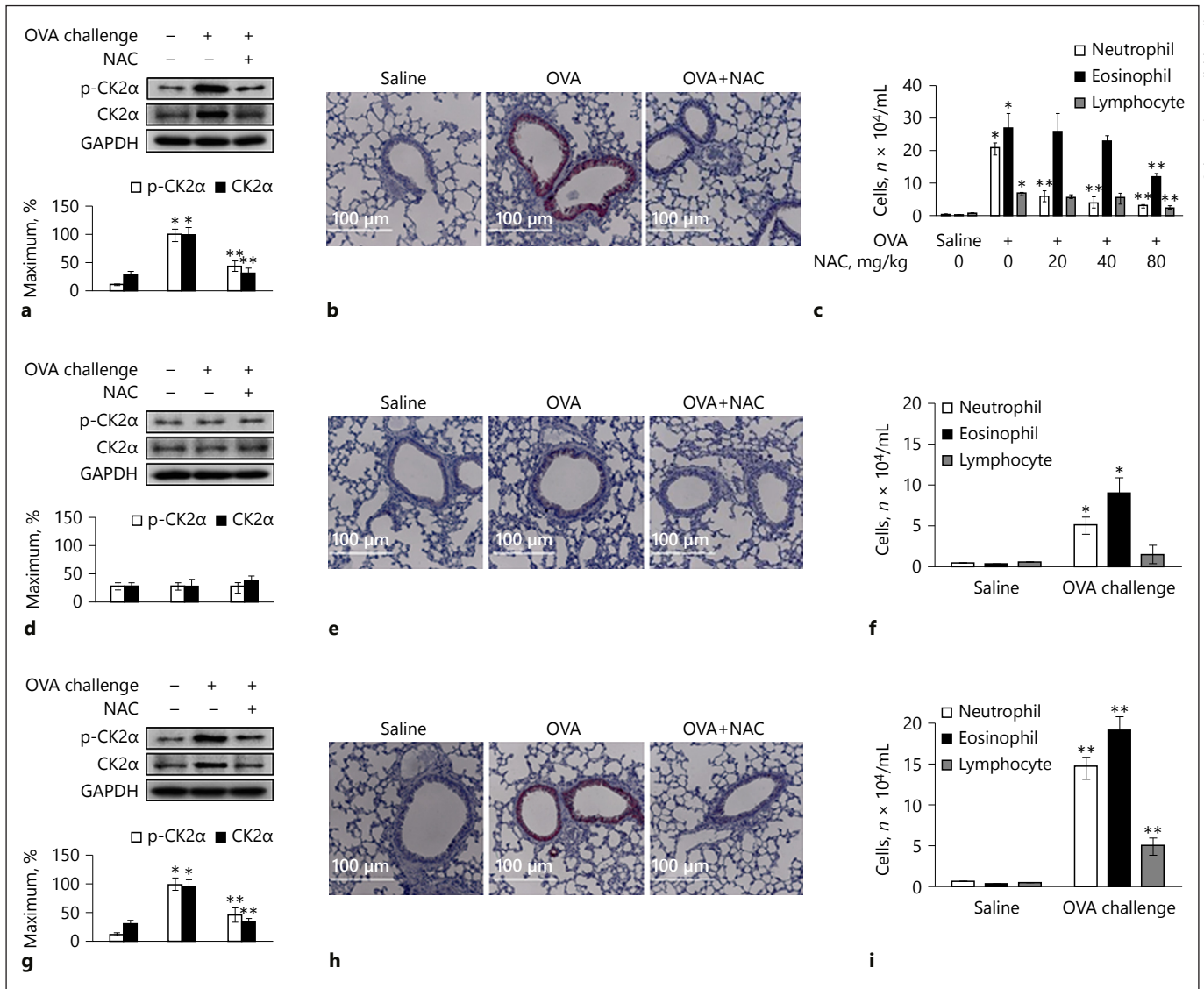


Fig. 3. Involvement of reactive oxygen species in CK2α phosphorylation and protein expression. Effects of NAC on CK2α phosphorylation and protein expression (**a**), immunohistochemistry of the CK2α protein (**b**), and number of neutrophils, eosinophils, and lymphocytes (**c**) at 8 h after challenge and eosinophils and lymphocytes at 36 h after challenge in BAL fluids in allergic inflammatory lungs of the littermates (C57BL/6 mice). CK2α phosphorylation and protein expression (**d**), immunohistochemistry of the CK2α protein (**e**), and number of neutrophils, eosinophils, and lymphocytes (**f**) in BAL fluids in allergic inflammatory lungs of NOX2^{-/-} mice. CK2α phosphorylation and protein expression (**g**), immu-

nohistochemistry of the CK2α protein (**h**), and number of neutrophils, eosinophils, and lymphocytes (**i**) in BAL fluids in allergic inflammatory lungs of NOX4^{-/-} mice. The lungs were removed at 30 min after challenge for CK2α phosphorylation and at 2 h after challenge for protein expression, respectively. NAC (20 mg/kg) was administered 1 h before the second airway challenge. Data are representative of 3–5 independent experiments with 3–5 mice per group per experiment. **a, c, d, g** **p* < 0.05 vs. control (saline) group and ***p* < 0.05 vs. OVA-challenged group. **f, i** **p* < 0.05 vs. saline group. OVA, ovalbumin.

kines (the BAL levels of KC showed a very similar pattern compared to those of MIP-2; data not shown) (Fig. 4c). Finally, we compared their ability to activate CK2α. IgE sensitization/OVA challenge induced neither CK2α

phosphorylation (Fig. 4d) nor protein expression (Fig. 4d, e). In contrast, IgG sensitization/OVA challenge induced CK2 phosphorylation (Fig. 4d) and protein expression (Fig. 4d, e).

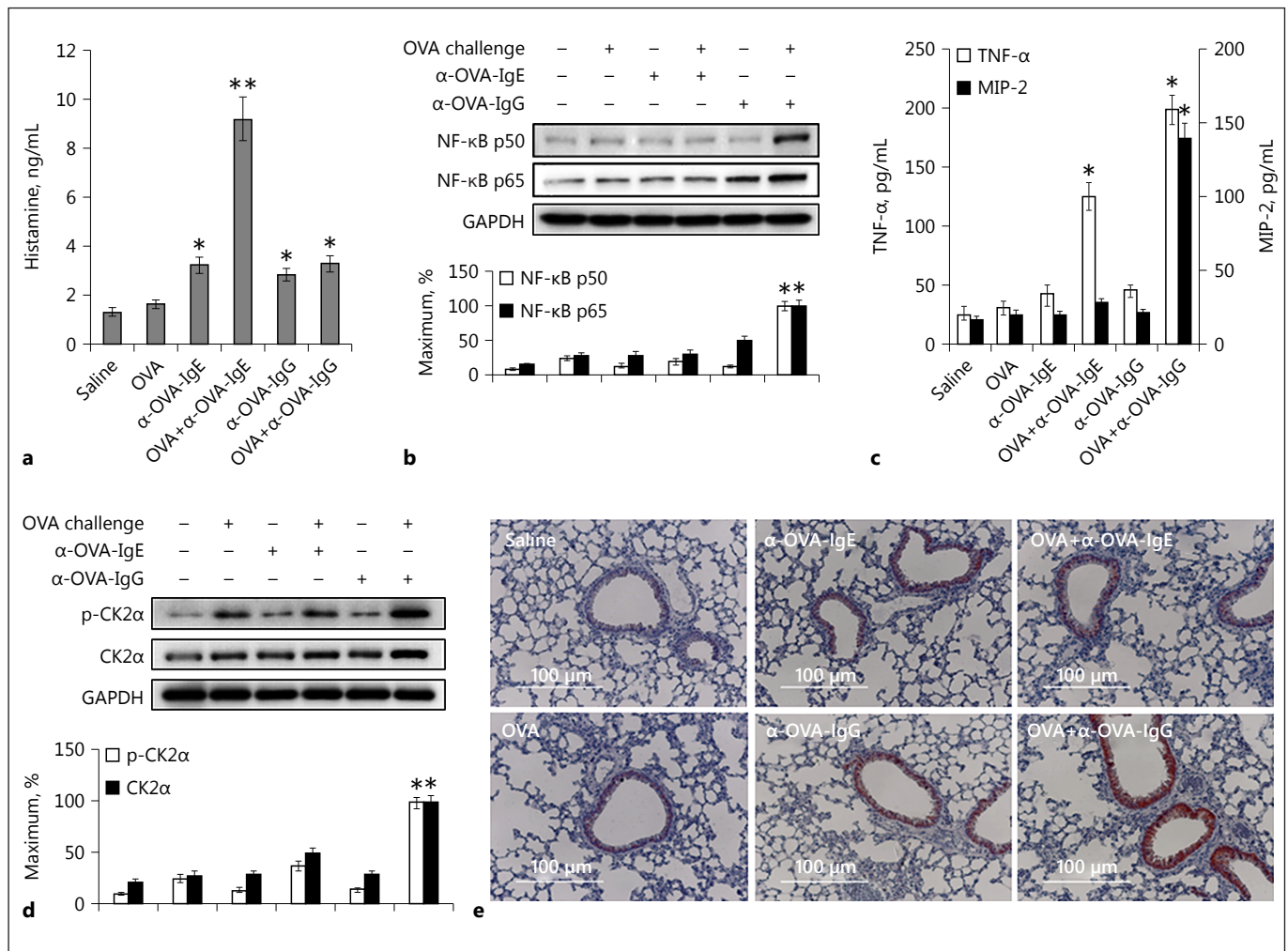


Fig. 4. Effects of passive transfer of anti-OVA IgE and anti-OVA IgG on nuclear factor (NF)-κB activation and CK2α phosphorylation and protein expression. **a** BAL levels of histamine at 30 min after challenge. **b** Western blot analysis of the p50 and p65 subunit of NF-κB at 1 h after challenge. **c** BAL levels of proinflammatory cytokines at 2 h after challenge. **d** Western blot analysis of CK2α phosphorylation and protein expression at 30 min and 2 h after

challenge, respectively. **e** Immunohistochemistry of the CK2α protein at 2 h after challenge. **a, c** Data represent the mean ± SEM of 3 independent experiments with 3–5 mice per group per experiment. * $p < 0.01$, ** $p < 0.05$. **b, d, e** Data are representative of 3–5 independent experiments with 3–5 mice per group per experiment. * $p < 0.05$ vs. OVA-challenged group. OVA, ovalbumin.

Discussion

A murine model of asthma in which the mice received i.p. immunization twice, followed by 2 bronchoprovocations with aerosolized OVA with a 1-week interval, has been previously developed [18]. This model seems to be a suitable tool to identify the cascade of events involved in an asthmatic reaction. Using the above-mentioned protocol, this study has demonstrated for the first time that CK2α plays a key role in NF-κB activation in asthmatic lungs.

The second airway challenge was shown to result in increased CK2α phosphorylation and protein expression. Western blot analysis demonstrated nuclear translocation of the p50 and p65 subunits of NF-κB, which was inhibited by pretreatment with the CK2α inhibitor TBBt. Consistent with the inhibition of NF-κB activation, TBBt inhibited BAL levels of the NF-κB-dependent proinflammatory cytokines (TNF-α, MIP-1, and KC) peaking at 1–2 h. Activation of NF-κB requires phosphorylation of IκB proteins by the IκB kinase (IKK) complex, which leads to IκB protein ubiquitination and its subsequent

degradation. This results in the release of cytoplasmic NF- κ B complexes, which then translocate to the nucleus and regulate the expression of target genes. CK2 α was shown to directly phosphorylate I κ B α at Ser 283, Ser 289, Ser 293, and Thr 291, which in turn facilitated the degradation of I κ B, thereby promoting the transcription of NF- κ B target genes [14, 15]. We also found that a second airway challenge resulted in increased phosphorylation of the p65 subunit at Ser 539, which was also inhibited by pretreatment with TBBt. Taken together, our data indicate that CK2 α plays an important role in NF- κ B activation in asthmatic lungs.

The mechanisms by which CK2 α activates NF- κ B in asthma remain unknown. A number of inflammatory mediators have been found to modulate the constitutive activity of CK2 α . For example, LPS [24], TNF- α [25], IL-1 [26], and transforming growth factor- β [27] stimulate CK2 α activity in *in vitro*. However, it is unlikely that cytokines and other proinflammatory agents were involved in CK2 α activation in this study, as CK2 α activation occurred prior to NF- κ B activation, which regulated the production of proinflammatory cytokines. It has been previously reported that LPS and proinflammatory cytokines produce ROS, which in turn activates CK2 α [19]. Consistent with these findings, we here demonstrated that NAC inhibited CK2 α phosphorylation and protein expression in allergic pulmonary inflammation. Furthermore, we showed that CK2 α phosphorylation and protein expression were impaired in NOX2^{-/-} but not in NOX4^{-/-} mice, indicating that ROS produced by NOX2 is importantly involved in CK2 α phosphorylation and protein expression.

Regarding the effect of ROS/CK2 α on inflammatory cell recruitment into the airways, bolus injection of TBBt (12.5 mg/kg) or NAC (20 mg/kg) at the concentrations at which they showed CK2 α inhibition (Fig. 1 and 3, respectively) resulted in a reduction of especially neutrophils in BAL fluids. To reduce eosinophil recruitment, 2 or 3 more concentrations of TBBt and NAC were required. Although we do not present the data here, we found that bolus injection of TBBt (12.5 mg/kg) or NAC (20 mg/kg) at 24 h postchallenge significantly reduced the number of eosinophils and lymphocytes, suggesting that: (1) there is a biphasic increase in ROS production in this murine model of pulmonary inflammation, and (2) the first and second peaks of ROS are involved in neutrophil and eosinophil recruitment, respectively. Further studies are required to address this issue.

As an upstream signaling pathway of ROS in CK2 α activation, we explored the initial signaling event in al-

lergic pulmonary inflammation, which is the ligation of Fc γ R in response to airway antigen challenge. For this purpose, we employed a passive asthma model in which the mice received *i.t.* instillation of OVA-specific IgE or IgG followed by an airway challenge with OVA. In this model, IgE sensitization/OVA challenge induced the release of histamine and TNF- α , indicating the induction of mast cell degranulation; however, it failed to induce NF- κ B activation. In contrast, IgG sensitization/OVA challenge released less histamine but induced NF- κ B activation and NF- κ B-dependent proinflammatory cytokines. These data are in agreement with a previous report [20] which showed that a single *i.t.* instillation of either IgE-immune complex (IC) or LO-ME-2 (an Ab that induces mast cell degranulation) is capable of releasing histamine but cannot induce NF- κ B activation in the lungs. In contrast, a single *i.t.* instillation of IgG-IC has been shown to induce NF- κ B activation, as well as NF- κ B-dependent proinflammatory molecules such as TNF- α and CXC chemokines [20]. Therefore, the model we developed in this study seems to be suitable for the study of passive asthma. Ligation of Fc γ R, rather than Fc ϵ R, has consistently exhibited the ability to induce NF- κ B activation. Using this model, we demonstrated that there is a correlation between CK2 α activation and NF- κ B activation, *i.e.*, IgG sensitization/OVA challenge has the ability to induce CK2 α activation, resulting in NF- κ B activation. However, IgE sensitization/OVA challenge has no such ability. IgG sensitization/OVA challenge-mediated CK2 α activation was blocked by pretreatment of NAC. Taken together, these findings suggest that generation of ROS by Fc γ R ligation is as upstream signaling event, and the prerequisite for CK2 α activation.

It is worthwhile to discuss the cell populations involved in ROS production and CK2 α and NF- κ B activation. NOX are the only enzymes whose primary function is to generate superoxide/ROS [28]. The NOX family consists of 7 members, *i.e.*, 5 NOX proteins (NOX1–5) and the 2 dual oxidases (Duox1 and Duox2). They are differentially expressed and regulated in various tissues with different subcellular localizations. NOX2 is the major isoform in phagocytes, such as neutrophils and macrophages. Thus, NOX2 contributes to many inflammatory diseases, whereas NOX4 is prominent in endothelial cells [29, 30]. Together with this, the fact that cells expressing Fc γ R produce ROS (Fig. 4) suggests that the residential pulmonary macrophages, which express both Fc γ R and NOX2, are the key cell population involved in ROS generation in murine asthma, rather than mast cells which

express FcεR1 in mice [31]. The sites of NF-κB activation in asthma could not be identified. However, given that airway epithelial cells are an important site for both CK2α (Fig. 1) and NF-κB activation [32], it seems that ROS derived from residential macrophages acts on airway epithelial cells to activate CK2α, which in turn induces NF-κB activation.

It has been reported that the mouse strain affects many aspect of asthma phenotypes [33, 34]. In this study, we used both BALB/c and C57BL/6 (NOX^{-/-}) mice. However, we found that there was no big difference in these mice in terms of CK2α expression and NF-κB activation.

In summary, we have demonstrated that FcγR/ROS/CK2α is the key inducer of NF-κB activation in a murine

model of asthma. Therefore, this study provides crucial information to help us to improve our understanding of the mechanisms underlying asthmatic inflammation.

Acknowledgements

This study was supported by the National Research Foundation of Korea (NRF) via a grant funded by the Korea government (MSIP) (No. 2008-0062279) and NRF-2014R1A2A2A01004207.

Disclosure Statement

The authors declare that they have no conflict of interests.

References

- Blackwell TS, Christman JW: The role of nuclear factor-κB in cytokine gene regulation. *Am J Respir Cell Mol Biol* 1997;17:3–9.
- Baldwin AS Jr: The NF-κB and IκB proteins: new discoveries and insights. *Annu Rev Immunol* 1996;14:649–683.
- Barnes PJ, Adcock IM: NF-κB: a pivotal role in asthma and a new target for therapy. *Trends Pharmacol Sci* 1997;18:46–50.
- Yang L, Cohn L, Zhang DH, Homer R, Ray A, Ray P: Essential role of nuclear factor κB in the induction of eosinophilia in allergic airway inflammation. *J Exp Med* 1998;188:1739–1750.
- Donovan CE, Mark DA, He HZ, Liou HC, Kobzik L, Wang Y, De Sanctis GT, Perkins DL, Finn PW: NF-κB/Rel transcription factors: c-Rel promotes airway hyperresponsiveness and allergic pulmonary inflammation. *J Immunol* 1999;163:6827–6833.
- Das J, Chen CH, Yang L, Cohn L, Ray P, Ray A: A critical role for NF-κB in GATA3 expression and TH2 differentiation in allergic airway inflammation. *Nat Immunol* 2001;2:45–50.
- Choi IW, Kim DK, Ko HM, Lee HK: Administration of antisense phosphorothioate oligonucleotide to the p65 subunit of NF-κB inhibits established asthmatic reaction in mice. *Int Immunopharmacol* 2004;4:1817–1828.
- Litchfield DW: Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. *Biochem J* 2003;369:1–15.
- Meggio F, Pinna LA: One-thousand-and-one substrates of protein kinase CK2? *FASEB J* 2003;17:349–368.
- Tawfic S, Yu S, Wang H, Faust R, Davis A, Ahmed K: Protein kinase CK2 signal in neoplasia. *Histol Histopathol* 2001;16:573–582.
- Guerra B, Issinger OG: Protein kinase CK2 in human diseases. *Curr Med Chem* 2008;15:1870–1886.
- Ahmad KA, Wang G, Unger G, Slaton J, Ahmed K: Protein kinase CK2 – a key suppressor of apoptosis. *Adv Enzyme Regul* 2008;48:179–187.
- Filhol O, Martiel JL, Cochet C: Protein kinase CK2: a new view of an old molecular complex. *EMBO Rep* 2004;5:351–355.
- McElhinny JA, Trushin SA, Bren GD, Chester N, Paya CV: Casein kinase II phosphorylates I kappa B alpha at S-283, S-289, S-293, and T-291 and is required for its degradation. *Mol Cell Biol* 1996;16:899–906.
- Schwarz EM, Van Antwerp D, Verma IM: Constitutive phosphorylation of IκBα by casein kinase II occurs preferentially at serine 293: requirement for degradation of free IκBα. *Mol Cell Biol* 1996;16:3554–3559.
- Wang D, Westerheide SD, Hanson JL, Baldwin AS Jr: Tumor necrosis factor-α-induced phosphorylation of RelA/p65 on Ser539 is controlled by casein kinase II. *J Biol Chem* 2000;275:32592–32597.
- Lee JH, Joo JH, Kim J, Lim HJ, Kim S, Curtiss L, Seong JK, Cui W, Yabe-Nishimura C, Bae YS: Interaction of NADPH oxidase 1 with Toll-like receptor 2 induces migration of smooth muscle cells. *Cardiovasc Res* 2013;99:483–493.
- Choi IW, Kim S, Kim YS, Ko HM, Im SY, Kim JH, You HJ, Lee YC, Lee JH, Park YM, Lee HK: TNF-α induces the late-phase airway hyperresponsiveness and airway inflammation through cytosolic phospholipase A₂ activation. *J Allergy Clin Immunol* 2005;116:537–543.
- Kim KJ, Cho KD, Jang KY, Kim HA, Kim HK, Lee HK, Im SY: Platelet-activating factor enhances tumour metastasis via the reactive oxygen species-dependent protein kinase casein kinase 2-mediated nuclear factor-κB activation. *Immunology* 2014;143:21–32.
- Lee CH, Kim YS, Kang NI, Lee YM, Kim KJ, Chai OH, Song CH, Kim HK, Im SY, Oh DK, Lee HK: IgG immune complex induces the recruitment of inflammatory cells into the airway and TNF-mediated late airway hyperresponsiveness via NF-κB activation in mice. *J Asthma* 2011;48:757–766.
- Kepley CL, Lauer FT, Oliver JM, Burchiel SW: Environmental polycyclic aromatic hydrocarbons, benzo(a) pyrene (BaP) and BaP-quinones, enhance IgE-mediated histamine release and IL-4 production in human basophils. *Clin Immunol* 2003;107:10–19.
- Suzuki Y, Yoshimaru T, Inoue T, Ra C: Discrete generations of intracellular hydrogen peroxide and superoxide in antigen-stimulated mast cells: reciprocal regulation of store-operated Ca²⁺ channel activity. *Mol Immunol* 2009;46:2200–2209.
- Franchini AM, Hunt D, Melendez JA, Drake JR: FcγR-driven release of IL-6 by macrophages requires NOX2-dependent production of reactive oxygen species. *J Biol Chem* 2013;288:25098–25108.
- Lodie TA, Savendra R Jr, Golenbock DT, Van Beveren CP, Maki RA, Fenton MJ: Stimulation of macrophages by lipopolysaccharide alters the phosphorylation state, conformation, and function of PU.1 via activation of casein kinase II. *J Immunol* 1997;158:1848–1856.
- Van Lint J, Agostinis P, Vandevoorde V, Haegeman G, Fiers W, Merlevede W, Vandenhede JR: Tumor necrosis factor stimulates serine/threonine protein kinases in Swiss 3T3 and L929 cells: implications of casein kinase-2 and extracellular signal-regulated kinases in the tumor necrosis factor signal transduction pathway. *J Biol Chem* 1992;267:25916–25921.

- 26 Parhar K, Morse J, Salh B: The role of protein kinase CK2 in intestinal epithelial cell inflammatory signaling. *Int J Colorectal Dis* 2007; 22:601–609.
- 27 Zdunek M, Silbiger S, Lei J, Neugarten J: Protein kinase CK2 mediates TGF- β 1-stimulated type IV collagen gene transcription and its reversal by estradiol. *Kidney Int* 2001;60:2097–2108.
- 28 Bedard K, Krause KH: The NOX family of ROS generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev* 2007;87: 245–313.
- 29 Coso S, Harrison I, Harrison CB, Vinh A, Sobey CG, Drummond GR, Williams ED, Selemidis S: NADPH oxidases as regulators of tumor angiogenesis: current and emerging concepts. *Antioxid Redox Signal* 2012;16: 1229–1247.
- 30 Brown DI, Griendling KK: Nox proteins in signal transduction. *Free Radic Biol Med* 2009;47:1239–1253.
- 31 de Andres B, Rakasz E, Hagen M, McCormik ML, Mueller AL, Elliot D, Metwali A, Sandor M, Britigan BE, Weinstock JV, Lynch RG: Lack of Fc- ϵ receptors on murine eosinophils: implication for the functional significance of elevated IgE and eosinophils in parasitic infections. *Blood* 1997;89:3826–3836.
- 32 Poynter ME, Irvin CG, Janssen-Heininger YM: Rapid activation of nuclear factor- κ B in airway epithelium in a murine model of allergic airway inflammation. *Am J Pathol* 2002; 160:1325–1334.
- 33 De Vooght V, Vanoirbeek JA, Luyts K, Haenen S, Nemery B, Hoet PH: Choice of mouse strain influences the outcome in a mouse model of chemical-induced asthma. *PLoS One* 2010;5:e12581–e12589.
- 34 Sahu N, Morales JL, Fowell D, August A: Modeling susceptibility versus resistance in allergic airway disease reveals regulation by Tec kinase I κ B. *PLoS One* 2010;5:e11348–e11356.