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Protein Kinase CK2/PTEN Pathway Plays a Key Role in Platelet-Activating Factor-Mediated Murine Anaphylactic Shock

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Platelet-activating factor (PAF) is a major mediator in the induction of fatal hypovolemic shock in murine anaphylaxis. This PAF-mediated effect has been reported to be associated with PI3K/Akt-dependent eNOS-derived NO. The phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is phosphatidylinositol phosphate phosphatase, which negatively controls PI3K by dephosphorylating the signaling lipid, phosphatidylinositol 3,4,5-triphosphate. In this study, we examined the possible involvement of PTEN in PAF-mediated anaphylactic shock. Induction of anaphylaxis or PAF injection resulted in a rapid decrease in PTEN activity, followed by increases in PI3K activity and phosphorylation of Akt and eNOS. Systemic administration of adenoviruses carrying PTEN cDNA (adenoviral PTEN), but not the control AdLacZ, not only attenuated anaphylactic symptoms, but also reversed anaphylaxis- or PAF-induced changes in PTEN and PI3K activities, as well as phosphorylation of Akt and eNOS. We found that the decreased PTEN activity was associated with PTEN phosphorylation, the latter effect being prevented by the protein kinase CK2 inhibitor, DMAT. DMAT also inhibited anaphylactic symptoms as well as the anaphylaxis- or PAF-mediated PTEN/PI3K/Akt/eNOS signaling cascade. CK2 activity was increased by PAF. The present data provide, as the key mechanism underlying anaphylactic shock, PAF triggers the upstream pathway CK2/PTEN, which ultimately leads to the activation of PI3K/Akt/eNOS. Therefore, CK2/PTEN may be a potent target in the control of anaphylaxis and other many PAF-mediated pathologic conditions. *The Journal of Immunology*, 2011, 186: 000–000.

Anaphylaxis is a life-threatening and systemic allergic reaction. Studies with mouse models demonstrate two pathways of systemic anaphylaxis: a classic pathway mediated by IgE, FcγRI, mast cells, histamine, and platelet-activating factor (PAF), and an alternative pathway mediated by IgG, FcγRIII, macrophages, and PAF (1). PAF is a major mediator in the induction of plasma extravasation by promoting vasodilation and increasing vascular permeability, the most important

contributor to fatal reactions in murine anaphylaxis (2). Such PAF-mediated effects have been reported to be associated with NO (3–5), a major endogenous regulator of vascular tone. NO directly affects blood flow by stimulating the release of the molecule cGMP, an arterial muscle relaxant, resulting in vasodilation (6).

PAF binds to a specific G-protein-coupled, seven-transmembrane receptor, the PAF-receptor, leading to hydrolysis of phosphatidylcholine, release of calcium from endoplasmic reticulum stores, and activation of protein kinase C, phospholipase C, phospholipase A₂, PI3K, and the MAPK cascade (7–11). In particular, activated PI3Ks generate phosphatidylinositol 3-phosphate (PIP₃) (3–5), leading to the recruitment and activation of Akt/protein kinase B, and activated Akt/protein kinase B phosphorylates several downstream targets including endothelial NO synthase (eNOS) (12–14). Recently, it has been reported that PI3K/Akt-dependent eNOS-derived NO is the principal vasodilator in PAF-induced and active anaphylaxis models (15).

The phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is PIP₃ phosphatase; it negatively controls PI3K-dependant signaling by dephosphorylating PIP₃ into phosphatidylinositol 2-phosphate (PIP₂). PTEN was initially identified as a tumor suppressor, and its loss or mutation is reported to be associated with the development of various cancers (16, 17). PTEN may exert this regulatory role by altering its own expression and activity in response to external stimuli such as inflammatory mediators or cytokines (18, 19). In addition, some studies have revealed decreases in PTEN expression by TNF (20) or cigarette smoking (21). Although researchers have recently identified the role of PTEN in allergic inflammation (22), the role of PTEN has not been studied in PAF-induced or anaphylactic shock.

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Abbreviations used in this article: AdPTEN, adenoviral PTEN; CK-2, casein kinase-2; DMAT, 2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole; eNOS, endothelial NO synthase; GSK-3, glycogen synthase kinase-3; Ht, hematocrit; PAF, platelet-activating factor; PIP₂, phosphatidylinositol 2-phosphate; PIP₃, phosphatidylinositol 3-phosphate; PTEN, phosphatase and tensin homologue deleted on chromosome 10.

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In this study, we examined the possible involvement of PTEN using a murine model of anaphylaxis. We found that the negatively modulating pathway of PI3K, involving protein kinase CK2/PTEN, played a key role in the pathogenesis of PAF-induced murine anaphylaxis.

Materials and Methods

Animals

Specific pathogen-free female C57BL/6 mice, purchased from Orient Bio (Gapyeong, Gyeonggi, Korea), were housed in a laminar flow cabinet throughout all experiments and maintained on standard laboratory chow ad libitum. Mice were ~7–8 wk old at the start of each experiment. All experimental animals used in this study were maintained under appropriate protocols approved by the Institutional Animal Care and Use Committee of the Chonbuk National University Medical School.

Reagents

OVA (grade V) and PAF (1-0-alkyl-2-acetyl-sn-glycerol-3-phosphorylcholine) were purchased from Sigma (St. Louis, MO). The PAF receptor antagonist, CV6209 (WAKO Chemical Company, Osaka, Japan) was administered i.p. (15) 10 min before challenge at a dose of 15 mg/kg. Control groups received the appropriate vehicles. CK2 inhibitor, dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole (DMAT), was purchased from Calbiochem (San Diego, CA) and administered i.p. at a dose of 25 mg/kg at 6 h before PAF injection. The GSK-3 inhibitor lithium chloride (LiCl) was purchased from Sigma and administered i.p. at a dose of 2.5 g/kg at 6 h before PAF injection. Another GSK-3 inhibitor, AR-A014418, was purchased from Sigma and administered i.p. at a dose of 9 µg/kg at 30 min before PAF injection. Wortmannin was administered i.p. at a dose of 1.5 mg/kg at 80 min before OVA-challenge or PAF injection. *Bordetella pertussis* was obtained from the National Institutes of Health (Seoul, Korea).

Measurement of body temperature

Rectal body temperature was measured to the nearest 0.1°C by inserting the probe of the thermocouple (BAT-12; Havard Apparatus, Holliston, MA) 20 mm into the rectum until a stable reading was obtained.

Construction of adenovirus and infection

Adenoviral PTEN (AdPTEN), the E1/E3-deleted replication-deficient recombinant adenovirus containing the wild type *PTEN* cDNA, was generated as described previously (22). In brief, *KpnI-XhoI* restriction fragments from pcDNA3/PTEN cDNA were ligated into the *KpnI-XhoI*-digested pShuttle-CMV. To create AdLacZ, a *BamHI-NotI* restriction fragment from pcDNA3.1/lacZ (Invitrogen, San Diego, CA) was ligated to the *SalI-NotI*-digested

pShuttle-CMV. Recombination into the pAdEasy-1 viral backbone and preparation of recombinant adenovirus were accomplished according to the manufacturer's instructions (Quantum Biotechnologies, Montreal, Canada).

After amplification, lysates containing clonal recombinant adenovirus were prepared from 150-mm culture dishes and purified by cesium chloride gradient centrifugation. Recovered viruses were separated into aliquots and stored at -20°C in 5 mM Tris (pH 8.0), 50 mM NaCl, 0.05% BSA, and 25% glycerol. Viruses were titered through serially diluted infections on QBI-293A cells and counting plaques under 0.3% agarose/10% FBS/1× DMEM overlay.

Induction of anaphylaxis

OVA-induced active systemic anaphylaxis was induced as described previously (23, 24). Briefly, mice were sensitized i.p. with 100 µg OVA, 2×10^9 *Bordetella pertussis*, and 1.0 mg alum. The mice were challenged with 30 µg OVA 21 d after initial sensitization. A passive anaphylaxis model was induced by PAF injection (0.3 µg i.v.). After the challenge injections were given, the mice were observed for 1 h, and signs of shock and the number of deaths were recorded as described previously (23, 24): grade 0, no sign of shock; grade 1, decreased spontaneous activity and loss of coordination with mild dyspnea; grade 2, no activity following whisker stimuli and progressive paresis beginning with the hind leg; grade 3, brief and violent convulsions, prostration, coma, and substantial recovery; and grade 4, fatal shock and death within 15–60 min.

Determination of hematocrit values

Late anaphylaxis was quantified by measuring increases in hematocrit (Ht) values as described previously (24). Mice were sacrificed 20 min after OVA challenge or PAF injection. The hearts were cut open, and small blood samples were collected into heparinized microhematocrit tubes (Proper Manufacturing, Long Island City, NY). Tubes were centrifuged for 5 min using a microhematocrit centrifuge (International Equipment, Needham Heights, MA), and the percentage of hematocrit change was expressed as [(Value at postchallenge - Value at prechallenge)/Value at prechallenge] × 100.

Western blotting

Lung tissues were homogenized with protease inhibitors and phosphatase inhibitors and prepared in protein extraction solution (PRO-PREP; iNtRON, Sungnam, Korea). Aliquots of each cytosolic extract, containing 80 µg of protein, were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes by electroblotting (Trans-Blot; Bio-Rad, Munchen, Germany). Nonspecific sites were blocked with 5% nonfat dry milk in TBST (25 mM Tris, pH 7.5, 150 mM NaCl, and 0.3% Tween 20) for 1 h at room temperature. Primary Abs used were as follows: anti-PTEN Ab (FL-403, Santa Cruz Biotechnology, Santa Cruz, CA), anti-phosphorylated PTEN (Cell Signaling Technology, Beverly, MA), anti-Akt (Cell Signaling

FIGURE 1. Decrease in PTEN activity during active anaphylaxis or by PAF injection. Lungs were removed at the indicated time points after the challenge injection of OVA (30 µg i.v.) (A, B) or PAF injection (0.3 µg i.v.) (C, D). The amount of PIP₂ produced by PTEN was determined by colorimetric detection. AdPTEN (3×10^9 PFU) or AdLacZ (3×10^9 PFU) was injected i.v. 2 d before OVA challenge (B) or PAF injection (D). Data represent the mean ± SD of three independent experiments, and each include five animals per group. ***p* < 0.01 versus normal control (0 min or no treatment group), ##*p* < 0.01 versus 5 min or positive control (OVA challenge).

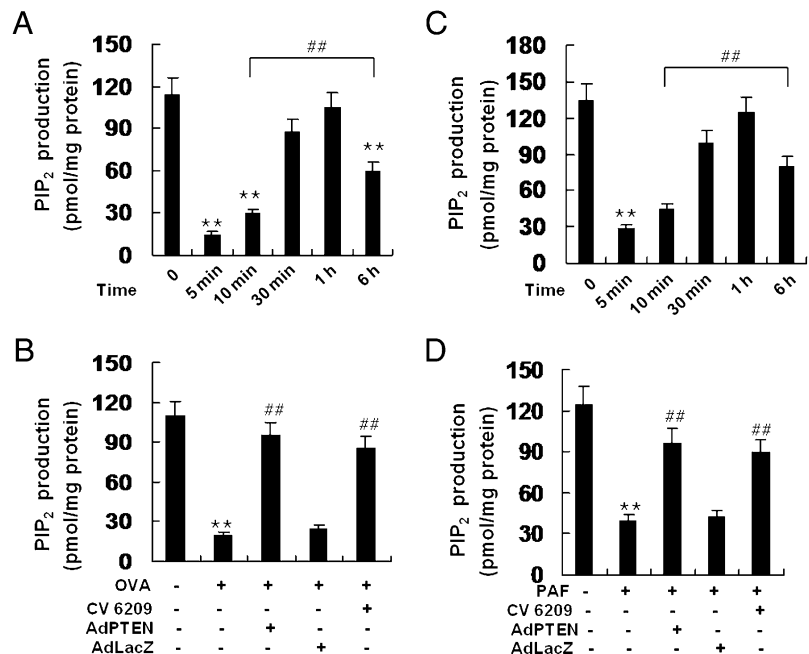
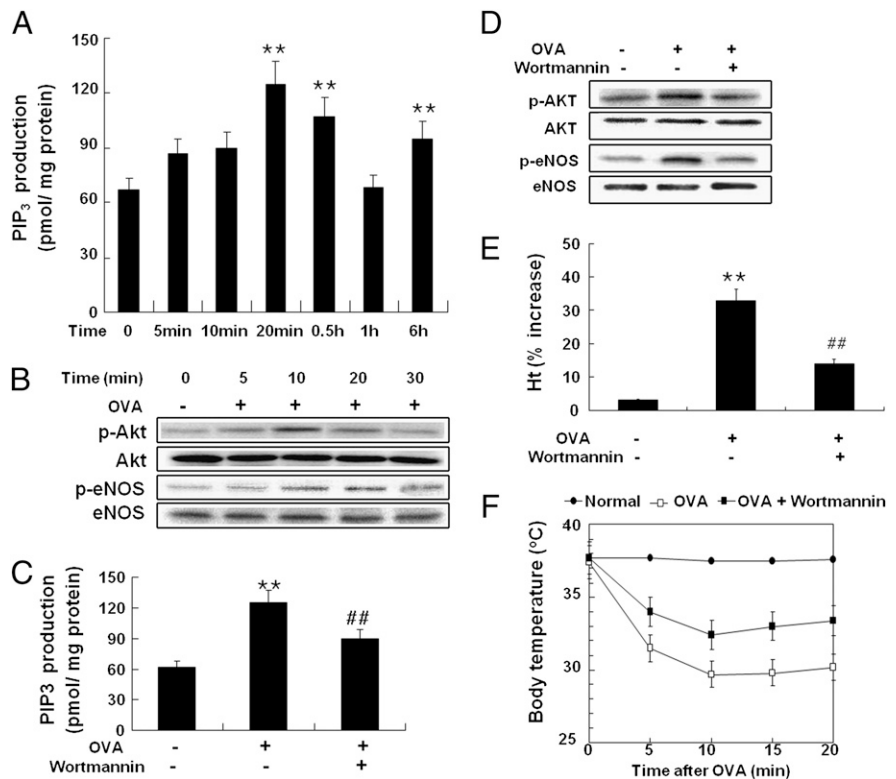


FIGURE 2. Involvement of the PI3K/Akt/eNOS pathway in the induction of anaphylaxis. Lungs were removed at indicated time points after OVA challenge (30 μ g i.v.). The enzyme activity of PI3K (A, C) was determined by quantifying PIP₃ production with a colorimetric assay. Protein levels and phosphorylation of Akt and eNOS were determined by Western blotting (B, D). Wortmannin (1.5 mg/kg) was administered i.p. 80 min before OVA-challenge. PIP₃ production (C), protein levels and phosphorylations of Akt and eNOS (D), and Ht values (E) were determined at 20 min after OVA challenge. Body temperature (F) was monitored over 20 min following OVA challenges. Data represent the mean $\pm$ SD of three independent experiments, and each includes five animals per group. * p < 0.05, ** p < 0.01 versus normal control or 0 min, ### p < 0.01 versus positive control (OVA challenge).



Technology) anti-phosphorylated Akt (Cell Signaling Technology), anti-eNOS (Cell Signaling Technology), and anti-phosphorylated eNOS Abs (Cell Signaling Technology). Blots were probed overnight at 4°C, and visualized using alkaline phosphatase-conjugated anti-rabbit IgG (Santa Cruz).

Measurement of PI3K enzyme activity in lung tissues

PI3K enzyme activity was measured using a PIP₃ competition enzyme immunoassay as described previously (25, 26). Homogenized lung tissues were incubated with PIP₂ substrate and reaction products were incubated with a PIP₃ detector protein. Reaction products were added to the PIP₃

coated microplate for competitive binding according to the manufacturer's protocol (Echelon, Salt Lake City, UT). Enzyme activity was expressed as amount of PIP₃ (pmol/ml) produced by equal amounts of tissue protein by colorimetric signal change.

Measurement of PTEN activity

Lung tissues were homogenized in the presence of protease inhibitors. Equal amounts of protein were incubated with the PIP₃ substrate according to the manufacturer's protocol (Echelon). PTEN mediated the PIP₃ conversion to PIP₂ in each sample. The PIP₂ detector and the HRP conjugate were added to the reaction products and colorimetric detection was used to

FIGURE 3. Inhibition of PI3K/Akt/eNOS pathways and anaphylactic symptoms by AdPTEN. The lungs were removed two days after i.v. injection of 3×10^9 PFUs of AdPTEN or AdLacZ (A). Mice were sacrificed at the indicated times (B) or 20 min (C, D) after OVA challenge (30 μ g i.v.). Ad PTEN (3×10^9 PFU) or AdLacZ was injected i.v. 2 d prior to the challenge. Body temperatures (E) were monitored over 20 min following OVA challenges. Data represent the mean $\pm$ SD of three independent experiments, and each includes five animals per group. * p < 0.05, ** p < 0.01 versus normal control, ## p < 0.01 versus positive control (OVA challenge group).

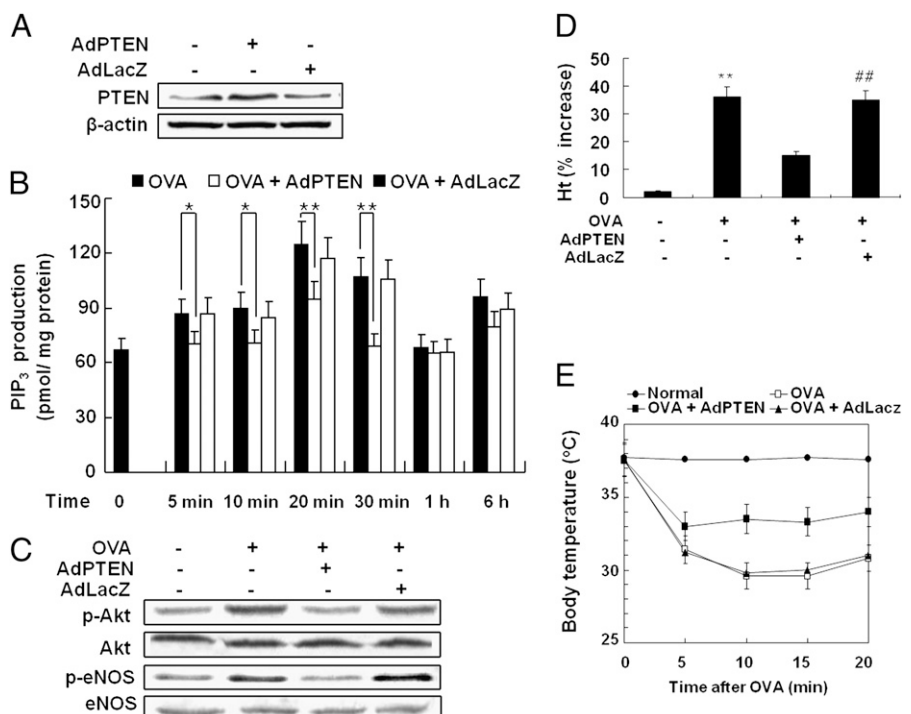


Table I. Administration of Wortmannin attenuates OVA-induced anaphylactic symptoms

Group	n	No. of Mice Showing Following Shock Score ^a				
		0	1	2	3	4
OVA challenge	10	0	0	0	6	4
OVA challenge + Wortmannin	10	1	3	5	1	0

Wortmannin was administered i.p. at a dose of 1.5 mg/kg at 80 min before OVA (30 µg i.v.) challenge.
^a0, No sign; 1, mild; 2, moderate; 3, severe; 4, death.

quantify the amount of PIP₂ produced by PTEN phosphatase activity. Results were compared with a standard curve.

Casein kinase II assay

To determine enzyme activity of casein kinase II, the phosphotransferase activity was measured according to the instructions described in commercial kit (casein kinase 2 assay kit; Millipore, Temecula, CA). This assay is based on phosphorylation of a specific substrate of CK II using the transfer of γ-[³²P] ATP by CK II. Protein samples were prepared in the same way with those in immunoblots; 10 µl assay dilution buffer I, 10 µl of the substrate peptide (200 µM final concentration), 10 µl protein kinase A inhibitor, and 10 µl diluted γ-[³²P] ATP were added to 200 µg protein. Reaction solution was incubated and agitated for 10 min at 30°C. The reaction was stopped by adding 20 µl of 40% trichloroacetic acid to each tube; 25-µl aliquots were transferred on numbered P81 paper squares allowing the radiolabeled substrate to bind to the papers for 30 s. The papers were immersed in 0.75% phosphoric acid and mixed gently on a rotator. After washing six times with 0.75% phosphoric acid and 1 min of washing in 20 ml of acetone, the paper squares were allowed to dry and then transferred to scintillation vials. After adding scintillation mixture, samples were read in scintillation counter.

Statistical analysis

Data were expressed as mean ± SD. Statistical comparison was performed with the one-way ANOVA followed by the Fisher's test. Significant dif-

ferences between the groups were calculated with the Student *t* test. Statistical significance was defined as *p* < 0.05.

Results

PAF decreases PTEN activity during anaphylaxis

PTEN activity was first examined in animals undergoing anaphylaxis. PIP₂ production by PTEN was monitored from whole lung tissues at fixed intervals subsequent to a challenge. Induction of anaphylaxis by OVA challenge resulted in rapid decreases in PTEN activity (Fig. 1A), which was apparent within the first 10 min of challenge. This effect normalized within 1 hour. The PAF antagonist CV 6209 blocked the decrease in PIP₂ (Fig. 1B), suggesting that PAF was involved in this process. PAF injection also resulted in decreased PIP₂ with similar kinetics to those seen during anaphylaxis (Fig. 1C). This loss was blocked again by CV 6209 (Fig. 1D), indicating that PAF decreases PTEN activity during anaphylaxis. For the recovery of PTEN activity, we used AdPTEN. Systemic administration of AdPTEN 2 d before challenge or PAF injection resulted in an inhibition of anaphylaxis-induced or PAF-induced decrease in PTEN activity (Fig. 1B, 1D). However, the control AdLacZ did not reveal such inhibition.

The PI3K/Akt signaling pathway was examined in mouse lungs during anaphylaxis to elucidate the pathophysiologic involvement of the downstream pathway of PTEN. After challenge injection, PIP₃ levels, which represent PI3K activity, continued to rise and ultimately revealed a twofold increase at 20 min, and were normalized within 1 h of challenge (Fig. 2A). Phosphorylations of Akt and eNOS were also increased within 10 min of OVA challenge injection (Fig. 2B). Pretreatment with the PI3K inhibitor Wortmannin significantly inhibited not only PIP₃ production (Fig. 2C) and the phosphorylations of Akt and eNOS (Fig. 2D), but also anaphylactic symptoms, such as hemoconcentration (Fig. 2E), body temperature (Fig. 2F), and anaphylactic signs (Table I).

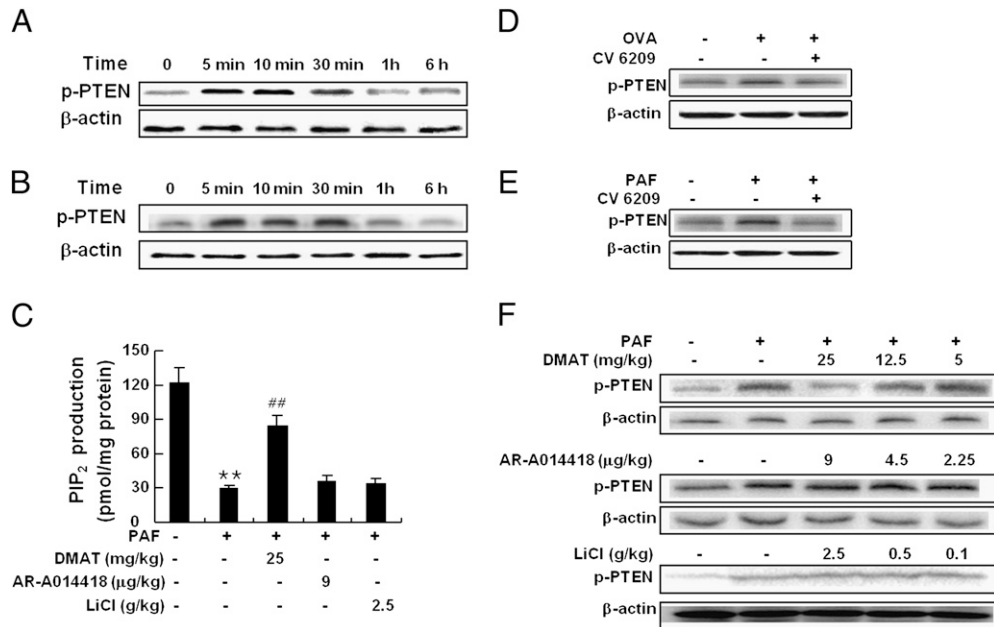


FIGURE 4. PTEN phosphorylation during anaphylaxis and its inhibition by CK2 inhibitor. Lungs were removed at the indicated times (A, B) or 5 min (C, D) after OVA challenge (30 µg i.v.) or PAF injection (0.3 µg i.v.). CV 6209 (15 mg/kg) was given i.p. 10 min before OVA challenge or PAF injection. PTEN phosphorylation inhibitors, DMAT and LiCl were administered 6 h prior to PAF injection, and AR-A014418 was administered 30 min prior to PAF injection. Protein levels of phosphorylated PTEN induced by OVA (A, D) and PAF (B, C, E, F) injections respectively were determined by Western blotting. The PIP₂ detector and HRP conjugate were added and the amount of PIP₂ produced by PTEN was determined by colorimetric assay (C). Data represents the mean ± SD of three experiments, and each includes five animals per group. ***p* < 0.01 versus normal control (no treatment group), ##*p* < 0.01 versus positive control (PAF injection group).

Table II. Administration of AdPTEN attenuates OVA-induced anaphylactic symptoms

Group	n	No. of Mice Showing Following Shock Score ^a				
		0	1	2	3	4
OVA challenge	10	0	0	0	5	5
OVA challenge + AdPTEN	10	0	4	6	0	0
OVA challenge + AdLacZ	10	0	0	0	8	2

AdPTEN (1.5×10^{10} i.v.) was administered 2 d before OVA (30 μ g i.v.) challenge.

^a0, No sign; 1, mild; 2, moderate; 3, severe; 4, death.

PTEN regulates PI3K/Akt signaling pathway during anaphylaxis

Using AdPTEN, we evaluated the direct role of PTEN in the PI3K/Akt signaling pathway and the induction of anaphylaxis. Mice were injected with 3×10^9 PFU of AdPTEN or AdLacZ i.v. Two days later, PTEN protein expression was evident in the lungs of mice that received AdPTEN, but not AdLacZ (Fig. 3A). AdPTEN inhibited PIP₃ production (Fig. 3B) and the phosphorylations of Akt and eNOS (Fig. 3C). In addition, AdPTEN significantly improved all the anaphylactic symptoms examined, such as hemoconcentration (Fig. 3D), body temperature (Fig. 3E), and anaphylactic signs (Table II). AdLacZ did not exhibit such effects.

CK2-induced PTEN phosphorylation occurs during anaphylaxis

PTEN activity has been reported to be negatively regulated by PTEN phosphorylation (27, 28); therefore we assessed whether PTEN phosphorylation occurred during anaphylaxis. OVA- or PAF-induced anaphylaxis resulted in PTEN phosphorylation with kinetics similar to those seen with PTEN activity (Fig. 4A, 4B), and the effect was inhibited by pretreatment with CV6209 (Fig. 4D, 4E). Similar kinetics in terms of PIP₂ levels as well as PTEN phosphorylation were induced by PAF injection and were inhibited by pretreatment with CV6209 (Fig. 4C). These data indicate that PAF-induced PTEN phosphorylation occurs in the early phase of anaphylaxis, which in turn results in decreased PTEN activity.

As the inducer of PTEN phosphorylation, we assessed the possible involvement of protein kinase CK2 (formerly known as casein kinase 2) and GSK-3 (formerly known as glycogen synthase kinase 3). PTEN is known to be phosphorylated by CK-2 on ser 370 and ser 385 (27) or by GSK-3 on ser 362 and thr 366 (29), reducing PTEN phosphatase activity (30). Both of the CK2 inhibitor (DMAT) and GSK3 inhibitors (AR-A014418, LiCl) at-

tenuated PAF-induced anaphylactic symptoms (Table III), but GSK3 inhibitors did not inhibit PTEN activity (Fig. 4C) and PTEN phosphorylation (Fig. 4F). As a result, we used only the CK2 inhibitor DMAT in the subsequent experiments. DMAT inhibited the PAF-induced increase in PTEN phosphorylation in a dose-dependent manner (Fig. 4F) and reversed the PAF-induced decrease in PTEN activity (Fig. 4C). These data suggest that CK2 is involved in the regulation of PTEN phosphorylation and activity.

PAF increases CK2 activity

We next observed the possibility that PAF might affect PTEN phosphorylation and activity via regulating CK2 activity during anaphylaxis. CK2 activity was increased immediately upon challenge injection and reached a peak at 5 or 10 min after challenge injection, gradually declining thereafter and returning to a basal level at 30 min (Fig. 5A). This increase was blocked by pretreatment with CV 6209. A similar kinetics of increase of CK2 activity was also observed by PAF injection, which was also blocked by CV 6209 (Fig. 5B). We also validated DMAT effectiveness by showing that it blocked anaphylaxis- or PAF-induced enhanced CK2 activity (Fig. 5A, 5B).

CK-2 is involved in the induction of anaphylaxis

Finally, we investigated the extent to which anaphylactic reactions are CK2 dependent in vivo. DMAT significantly improved the anaphylactic symptoms, such as hemoconcentration (Fig. 6A), body temperature (Fig. 6C), and anaphylactic signs (data not shown). Furthermore, DMAT inhibited anaphylaxis-induced increases in PI3K activity (Fig. 6B) and the phosphorylation of Akt and eNOS (Fig. 6D).

Discussion

PAF is a potent vasodilator whose activity can lead to fatal hypovolemic shock during murine anaphylaxis. Cauwels et al. (15) recently revealed PI3K/Akt-dependent eNOS-derived NO as the principal vasodilator in PAF-induced anaphylactic shock. In this study, we found that the PI3K/Akt signaling pathway is critical in the induction of murine anaphylaxis. This finding was accomplished by demonstrating that the PI3K inhibitor Wortmannin significantly inhibited PIP₃ production and the phosphorylations of Akt and eNOS in addition to improving the anaphylactic symptoms examined.

In this study, we have identified CK2/PTEN as the novel negatively modulating pathway of PI3K, involved in PAF-induced systemic anaphylaxis. Induction of anaphylaxis by OVA chal-

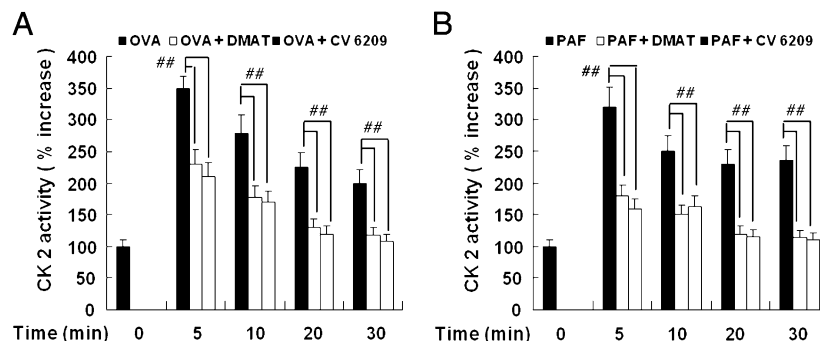


FIGURE 5. PAF-induced increase in CK2 activity during anaphylaxis. Lungs were removed at the indicated times after OVA challenge (30 μ g i.v.; A) or PAF injection (0.3 μ g i.v.; B). CV 6209 (15 mg/kg) was given i.p. 10 min before OVA challenge or PAF injection. Enzyme activity of CK2 was determined by detecting γ -[³²P] incorporated into substrates. DMAT (25 mg/kg) was given i.p. 6 h before challenge or PAF injection. Data represent the mean $\pm$ SD of three experiments, and each includes five animals per group. ** p < 0.01 versus 0 min, ## p < 0.01 versus positive control group (OVA challenge or PAF injection group).

Table III. Administration of CKII inhibitor attenuates PAF-induced anaphylactic symptoms

Group	n	No. of Mice Showing Following Shock Score ^a				
		0	1	2	3	4
PAF challenge	10	0	0	2	8	0
PAF challenge + DMAT	10	0	4	5	1	0
PAF challenge + AR-A014418	10	0	4	4	2	0
PAF challenge + LiCl	10	0	4	4	2	0

DMAT (25 mg/kg, 6 h before, i.p.) or AR-A014418 (9 μ g/kg, 30 min before, i.p.) or LiCl (2.5 g/kg, 6 h before, i.p.) was administered at the indicated time point before PAF (0.3 μ g/mouse, i.v.) challenge.

^a0, No sign; 1, mild; 2, moderate; 3, severe; 4, death.

challenge resulted in rapid decrease of PTEN activity, which was blocked by the PAF antagonist CV 6209. PAF injection also resulted in decreased PTEN activity, indicating that PAF is responsible for the decrease in PTEN activity during anaphylaxis. We showed that the decreased PTEN activity had a role in the induction of anaphylaxis by demonstrating that systemic administration of AdPTEN not only restored anaphylaxis-induced or PAF-induced decreases in PTEN activity, but also improved all the anaphylactic symptoms. Furthermore, AdPTEN inhibited PIP₃ production as well as the phosphorylation of Akt and eNOS, indicating that PI3K/Akt pathway is under the influence of PTEN activity.

How does PAF decrease PTEN activity? The PTEN protein consists of an N-terminal phosphatase domain, a lipid-binding C2 domain, and a 50-amino acid C-terminal domain (the "tail") of unknown function. Vazquez et al. (28) reported that the PTEN tail is necessary for maintaining protein stability, and that it also acts to inhibit PTEN function. Furthermore, tail-dependent regulation of stability is linked to the phosphorylation of three residues (S380, T382, and T383) within the tail. Therefore, phosphoryla-

tion of the tail is likely to decrease PTEN function. Given this information, we hypothesized that PAF decreases PTEN activity through its phosphorylation. As expected, we found that PAF strongly induced PTEN phosphorylation of which time kinetics corresponded with those of PTEN activity. These data suggested that PAF inhibited PTEN activity by inducing PTEN phosphorylation.

It has been reported that PTEN is phosphorylated by CK-2 on Ser370 and Ser385 (27, 29, 30). Therefore, we investigated whether inhibition of CK-2 could diminish the negative effect of PAF on PTEN. DMAT, a specific CK2 inhibitor, prevented the PAF-induced decrease of PTEN activity and attenuated anaphylactic responses, suggesting that CK2 is involved in the PAF-induced PTEN regulation. Importantly, we also showed that PAF rapidly increases CK2 activity during anaphylaxis, by demonstrating that 1) CK2 activity was increased immediately upon challenge injection and reached a peak after 5 or 10 min, an effect which was blocked by pretreatment with CV 6209, and 2) a similar kinetics of increase of CK2 activity was observed by PAF injection. In addition, our findings that DMAT not only attenuated anaphylactic symptoms, but also inhibited the anaphylaxis-induced increases in PI3K activity as well as phosphorylation of Akt and eNOS, suggest that CK2 is one of the upstream pathway of PI3K in the induction of PAF-induced anaphylaxis. PTEN is also known to be phosphorylated by GSK-3, but GSK-3 inhibitor did not inhibit the PTEN phosphorylation and activity, although it had a preventive effect on anaphylactic symptoms. Given that GSK-3 can phosphorylate many other molecules besides PTEN, it can be involved in a variety of signaling pathways that have critical roles in regulating extravasation, decreased vascular tone, and allergic responses of inflammatory cells during anaphylactic shock. We assumed that there should be another pathway via which GSK-3 regulates anaphylactic response, and it could be a candidate for the therapeutic target molecule. The mechanism by

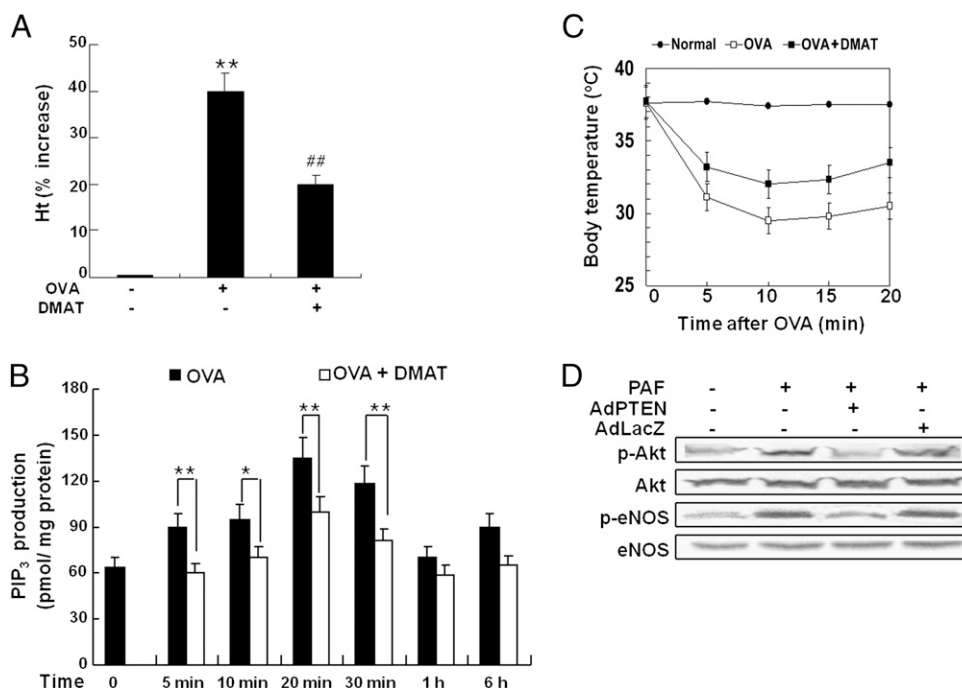


FIGURE 6. Involvement of CK2 in the induction of anaphylaxis. Lungs of mice were collected at 20 min (A, D) or the indicated times (C) after PAF injection (0.3 μ g i.v.) or OVA challenge (30 μ g i.v.). DMAT (25 mg/kg) was given i.p. 6 h before PAF injection or OVA challenge. The enzyme activities of PI3K (C) were determined by quantifying PIP₃ production with colorimetric assay. Protein levels and phosphorylations of Akt and eNOS were determined by Western blotting (D). Data represents the mean $\pm$ SD of three experiments, and each includes five animals per group. ** p < 0.01 versus normal control, # p < 0.05, ## p < 0.01 versus positive control group (OVA challenge or PAF injection group).

which GSK-3 can act on anaphylaxis remains to be further investigated.

CK2 is a highly conserved and ubiquitous protein Ser/Thr kinase localized in the cell nucleus and cytoplasm. Protein kinase activity of CK2 has been known to affect cellular activities involved in growth, proliferation, and apoptosis (31, 32). CK2 has a critical role in the development of various types of cancer, including that of the prostate and breast, through the activation of β -catenin in Wnt signaling (33). CK2 has been associated with specific neural functions by controlling neuronal differentiation at various levels, including neurogenesis and the formation of synaptic connections (34). Accumulating data also indicate that CK2 is involved in critical diseases of the CNS, such as Alzheimer's and Parkinson's diseases (35). CK2 also regulates inflammation by affecting key transcription factors such as NF- κ B. CK2 has been found to phosphorylate I κ B α at a number of sites, resulting in the degradation of the protein and the subsequent activation of NF- κ B, with implications to inflammation (36, 37). Thus, regulation of CK2 is regarded as a promising target for interfering with the progression of these diseases.

Although little is known about the relationship between PAF and CK2, Silva-Neto et al. (38) concurred with our data in reporting that PAF activated CK2 in protozoan parasites. In this regard, we present another example of the relationship between PAF and CK2 in the pathogenesis of anaphylaxis. Further study is required to address the underlying mechanism of how PAF activates CK2. PAF is released immediately or produced in response to inflammatory stimuli by a variety of inflammatory cells such as platelets, leukocytes, macrophages, and mast cells (39, 40). We have reported that PAF functions as a proximal mediator in the initiation of a diverse series of inflammatory reactions through the activation of the transcription factor, NF- κ B (40–44). However, the fact that PAF is able to activate CK2 would help delineate the exact biological roles of PAF. Furthermore, given that more than 300 substrates of CK2 have been identified to date, we can speculate that PAF may have more biologic activities than initially expected.

In summary, our data identify CK2/PTEN as a negatively modulating pathway of the known PI3K/eNOS pathway with respect to PAF-mediated anaphylactic shock. Therefore, CK2/PTEN may be a potent target in the control of anaphylaxis and other many PAF-mediated pathologic conditions.

Disclosures

The authors have no financial conflicts of interest.

References

- Finkelman, F. D. 2007. Anaphylaxis: lessons from mouse models. *J. Allergy Clin. Immunol.* 120: 506–515, quiz 516–517.
- Munoz, J., and R. K. Bergman. 1965. Mechanism of Anaphylactic Death in the Mouse. *Nature* 205: 199–200.
- Takano, H., T. Yoshikawa, T. Yamaguchi, H. Hongo, T. Fujii, Y. Boku, N. Yoshida, H. Yamamoto, Y. Nakahashi, R. Yanagisawa, and M. Kondo. 1997. Effects of aminoguanidine on systemic inflammatory response syndrome induced by platelet activating factor and by lipopolysaccharide in rats. *Eur. J. Pharmacol.* 323: 107–110.
- Wang, H. G., T. Shibamoto, Y. Matsuda, and S. Koyama. 1998. The role of endogenous nitric oxide in the sympathetic and hemodynamic response to platelet activating factor-induced hypotension in anesthetized dogs. *Shock* 9: 58–64.
- Yoshikawa, T., H. Takano, H. Yamamoto, Y. Nakahashi, T. Yamaguchi, T. Arimoto, and M. Kondo. 1997. Role of superoxide and nitric oxide in platelet-activating factor-induced acute lung injury, hypotension, and mortality in rats. *Crit. Care Med.* 25: 286–292.
- Ortega Mateo, A., Amaya Alexandre de Artiñano. 2000. Nitric oxide reactivity and mechanisms involved in its biological effects. *Pharmacol. Res.* 42: 421–427.
- Nakamura, M., Z. Honda, T. Izumi, C. Sakanaka, H. Mutoh, M. Minami, H. Bito, Y. Seyama, T. Matsumoto, M. Noma, et al. 1991. Molecular cloning and expression of platelet-activating factor receptor from human leukocytes. *J. Biol. Chem.* 266: 20400–20405.
- Valone, F. H. 1988. Identification of platelet-activating factor receptors in P388D1 murine macrophages. *J. Immunol.* 140: 2389–2394.
- Mauco, G., H. Chap, and L. Douste-Blazy. 1983. Platelet activating factor (PAF-acether) promotes an early degradation of phosphatidylinositol 4,5-bisphosphate in rabbit platelets. *FEBS Lett.* 153: 361–365.
- Nishizuka, Y. 1986. Studies and perspectives of protein kinase C. *Science* 233: 305–312.
- Shimizu, T., M. Mori, H. Bito, C. Sakanaka, S. Tabuchi, M. Aihara, and K. Kume. 1996. Platelet-activating factor and somatostatin activate mitogen-activated protein kinase (MAP kinase) and arachidonate release. *J. Lipid Mediat. Cell Signal.* 14: 103–108.
- Franke, T. F., S. I. Yang, T. O. Chan, K. Datta, A. Kazlauskas, D. K. Morrison, D. R. Kaplan, and P. N. Tsichlis. 1995. The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. *Cell* 81: 727–736.
- Dimmeler, S., I. Fleming, B. Fisslthaler, C. Hermann, R. Busse, and A. M. Zeiher. 1999. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* 399: 601–605.
- Fulton, D., J. P. Gratton, T. J. McCabe, J. Fontana, Y. Fujio, K. Walsh, T. F. Franke, A. Papadopoulos, and W. C. Sessa. 1999. Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. *Nature* 399: 597–601.
- Cauwels, A., B. Janssen, E. Buys, P. Sips, and P. Brouckaert. 2006. Anaphylactic shock depends on PI3K and eNOS-derived NO. *J. Clin. Invest.* 116: 2244–2251.
- Karoui, M., C. Tresallet, C. Julie, U. Zimmermann, F. Staroz, A. Brams, C. Muti, C. Boulard, A. M. Robreau, H. Puy, et al. 2004. Loss of heterozygosity on 10q and mutational status of PTEN and BMP1A in colorectal primary tumours and metastases. *Br. J. Cancer* 90: 1230–1234.
- Poetsch, M., G. Lorenz, and B. Kleist. 2002. Detection of new PTEN/MMAC1 mutations in head and neck squamous cell carcinomas with loss of chromosome 10. *Cancer Genet. Cytogenet.* 132: 20–24.
- Hou, R., J. Zhang, T. Yin, H. Cao, N. Zhang, X. Li, L. Wang, Y. Xing, D. Li, and Q. Ji. 2010. Upregulation of PTEN by peroxynitrite contributes to cytokine-induced apoptosis in pancreatic beta-cells. *Apoptosis* 15: 877–886.
- Koul, D., Y. Yao, J. L. Abbruzzese, W. K. Yung, and S. A. Reddy. 2001. Tumor suppressor MMAC/PTEN inhibits cytokine-induced NF κ B activation without interfering with the IkappaB degradation pathway. *J. Biol. Chem.* 276: 11402–11408.
- Kim, S., C. Domon-Dell, J. Kang, D. H. Chung, J. N. Freund, and B. M. Evers. 2004. Down-regulation of the tumor suppressor PTEN by the tumor necrosis factor- α /nuclear factor- κ B (NF- κ B)-inducing kinase/NF- κ B pathway is linked to a default IkappaB- α autoregulatory loop. *J. Biol. Chem.* 279: 4285–4291.
- Lee, S. Y., E. J. Kang, G. Y. Hur, K. H. Jung, H. C. Jung, S. Y. Lee, J. H. Kim, C. Shin, K. H. In, K. H. Kang, et al. 2006. Peroxisome proliferator-activated receptor- γ inhibits cigarette smoke solution-induced mucin production in human airway epithelial (NCI-H292) cells. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 291: L84–L90.
- Kwak, Y. G., C. H. Song, H. K. Yi, P. H. Hwang, J. S. Kim, K. S. Lee, and Y. C. Lee. 2003. Involvement of PTEN in airway hyperresponsiveness and inflammation in bronchial asthma. *J. Clin. Invest.* 111: 1083–1092.
- Park, J. S., I. H. Choi, D. G. Lee, S. S. Han, T. Y. Ha, J. H. Lee, W. H. Lee, Y. M. Park, and H. K. Lee. 1997. Anti-IL-4 monoclonal antibody prevents antibiotics-induced active fatal anaphylaxis. *J. Immunol.* 158: 5002–5006.
- Choi, I. H., Y. M. Shin, J. S. Park, M. S. Lee, E. H. Han, O. H. Chai, S. Y. Im, T. Y. Ha, and H. K. Lee. 1998. Immunoglobulin E-dependent active fatal anaphylaxis in mast cell-deficient mice. *J. Exp. Med.* 188: 1587–1592.
- Kim, K. M., and Y. J. Lee. 2005. Amiloride augments TRAIL-induced apoptotic death by inhibiting phosphorylation of kinases and phosphatases associated with the PI3K-Akt pathway. *Oncogene* 24: 355–366.
- Lee, K. S., S. R. Kim, S. J. Park, H. K. Lee, H. S. Park, K. H. Min, S. M. Jin, and Y. C. Lee. 2006. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) reduces vascular endothelial growth factor expression in allergen-induced airway inflammation. *Mol. Pharmacol.* 69: 1829–1839.
- Miller, S. J., D. Y. Lou, D. C. Seldin, W. S. Lane, and B. G. Neel. 2002. Direct identification of PTEN phosphorylation sites. *FEBS Lett.* 528: 145–153.
- Vazquez, F., S. Ramaswamy, N. Nakamura, and W. R. Sellers. 2000. Phosphorylation of the PTEN tail regulates protein stability and function. *Mol. Cell. Biol.* 20: 5010–5018.
- Al-Khouri, A. M., Y. Ma, S. H. Togo, S. Williams, and T. Mustelin. 2005. Co-operative phosphorylation of the tumor suppressor phosphatase and tensin homologue (PTEN) by casein kinases and glycogen synthase kinase 3 β . *J. Biol. Chem.* 280: 35195–35202.
- Torres, J., and R. Pulido. 2001. The tumor suppressor PTEN is phosphorylated by the protein kinase CK2 at its C terminus. Implications for PTEN stability to proteasome-mediated degradation. *J. Biol. Chem.* 276: 993–998.
- Wang, G., K. A. Ahmad, and K. Ahmed. 2006. Role of protein kinase CK2 in the regulation of tumor necrosis factor-related apoptosis inducing ligand-induced apoptosis in prostate cancer cells. *Cancer Res.* 66: 2242–2249.
- Litchfield, D. W. 2003. Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. *Biochem. J.* 369: 1–15.
- Seldin, D. C., E. Landesman-Bollag, M. Farago, N. Currier, D. Lou, and I. Dominguez. 2005. CK2 as a positive regulator of Wnt signalling and tumorigenesis. *Mol. Cell. Biochem.* 274: 63–67.

34. Blanquet, P. R. 2000. Casein kinase 2 as a potentially important enzyme in the nervous system. *Prog. Neurobiol.* 60: 211–246.
35. Guerra, B., and O. G. Issinger. 2008. Protein kinase CK2 in human diseases. *Curr. Med. Chem.* 15: 1870–1886.
36. McElhinny, J. A., S. A. Trushin, G. D. Bren, N. Chester, and C. V. Paya. 1996. 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.* 16: 899–906.
37. Shen, J., P. Channavajhala, D. C. Seldin, and G. E. Sonenshein. 2001. Phosphorylation by the protein kinase CK2 promotes calpain-mediated degradation of IkappaBalpha. *J. Immunol.* 167: 4919–4925.
38. Silva-Neto, M. A., A. B. Carneiro, D. P. Vieira, R. D. Mesquita, and A. H. Lopes. 2002. Platelet-activating factor (PAF) activates casein kinase 2 in the protozoan parasite *Herpetomonas muscarum muscarum*. *Biochem. Biophys. Res. Commun.* 293: 1358–1363.
39. Braquet, P., L. Touqui, T. Y. Shen, and B. B. Vargaftig. 1987. Perspectives in platelet-activating factor research. *Pharmacol. Rev.* 39: 97–145.
40. Han, S. J., J. H. Choi, H. M. Ko, H. W. Yang, I. W. Choi, H. K. Lee, O. H. Lee, and S. Y. Im. 1999. Glucocorticoids prevent NF-kappaB activation by inhibiting the early release of platelet-activating factor in response to lipopolysaccharide. *Eur. J. Immunol.* 29: 1334–1341.
41. Im, S. Y., S. J. Han, H. M. Ko, J. H. Choi, S. B. Chun, D. G. Lee, T. Y. Ha, and H. K. Lee. 1997. Involvement of nuclear factor-kappa B in platelet-activating factor-mediated tumor necrosis factor-alpha expression. *Eur. J. Immunol.* 27: 2800–2804.
42. Choi, J. H., W. J. Chung, S. J. Han, H. B. Lee, I. W. Choi, H. K. Lee, K. Y. Jang, D. G. Lee, S. S. Han, K. H. Park, and S. Y. Im. 2000. Selective involvement of reactive oxygen intermediates in platelet-activating factor-mediated activation of NF-kappaB. *Inflammation* 24: 385–398.
43. Choi, I. W., Y. S. Kim, D. K. Kim, J. H. Choi, K. H. Seo, S. Y. Im, K. S. Kwon, M. S. Lee, T. Y. Ha, and H. K. Lee. 2003. Platelet-activating factor-mediated NF-kappaB dependency of a late anaphylactic reaction. *J. Exp. Med.* 198: 145–151.
44. Seo, K. H., H. S. Lee, B. Jung, H. M. Ko, J. H. Choi, S. J. Park, I. H. Choi, H. K. Lee, and S. Y. Im. 2004. Estrogen enhances angiogenesis through a pathway involving platelet-activating factor-mediated nuclear factor-kappaB activation. *Cancer Res.* 64: 6482–6488.