INTRODUCTION

Anaphylaxis is an immediate, immunoglobulin E (IgE)-mediated hypersensitivity reaction resulting from the sudden release of mast cell- and basophil-derived chemical mediators such as histamine, serotonin, newly synthesized lipid-derived mediators, such as prostaglandin D₂ (PGD₂) and 5-lipoxygenase (5-LO) dependent leukotriene C₄ (LTC₄) generation dose-dependently in BMMCs. To probe the mechanism involved, we assessed the effects of curcumin on the phosphorylation of Syk and its downstream signal molecules. Curcumin inhibited intracellular Ca²⁺ influx via phospholipase Cγ₁ (PLCγ₁) activation and the phosphorylation of mitogen-activated protein kinases (MAPKs) and the nuclear factor-κB (NF-κB) pathway. Furthermore, the oral administration of curcumin significantly attenuated IgE/Ag-induced PSA, as determined by serum LTC₄, PGD₂, and histamine levels. Taken together, this study shows that curcumin offers a basis for drug development for the treatment of allergic inflammatory diseases.

Key Words: Curcumin, Mast cell, Prostaglandin D₂, Leukotriene C₄, Mitogen activated protein kinase, Passive systemic anaphylaxis
Curcumin (diferuloyl methane) is a major constituent of the rhizome of Curcuma longa, and is used traditionally to treat inflammation, gastrointestinal disorders, hepatic disorders, diabetic wounds, skin wounds, rheumatism, sinusitis, and other disorders (Ammon and Wahl, 1991). Furthermore, scientific studies have shown that curcumin inhibits histamine release and the secretions of tumor necrosis factor-α (TNF-α) and interleukin-4 (IL-4) from mast cells triggered by IgE, calcium ionophore A23187, or compound 48/80 (Suzuki et al., 2005; Lee et al., 2008; Choi et al., 2010). Curcumin has also been reported to inhibit IgE-induced type I hypersensitivity and ovalbumin-induced airway hyperreactivity (Yano et al., 2000; Ram et al., 2003; Lee et al., 2008), and to inhibit COX-2 gene expression in phorbol ester-treated human gastrointestinal epithelial cells and mouse skin (Chun et al., 2003; Ricciotti and FitzGerald, 2011) and in vitro lipoxigenase and cyclooxygenase activities in mouse epidermis (Huang et al., 1991). However, the effect of curcumin on IgE/Ag-induced COX-2 dependent PGD2, and 5-LO dependent LTC4 generation in mast cells and IgE-mediated systemic anaphylactic response have not been well investigated.

In this study, we evaluated the effects of curcumin on the generation of eicosanoid (PGD2, and LTC4) in FcεRI-induced mast cells and on passive systemic anaphylaxis (PSA) response in mice.

**MATERIALS AND METHODS**

**Plant material**

Curcumin was isolated from the ethyl acetate fraction of a methanol extract of the rhizome of Curcuma longa, as described previously (Kuichi et al., 1993), and produced a single TLC spot and had a HPLC determined purity of >99.5%. Curcumin was prepared by dissolving it in dimethyl sulfoxide (DMSO). The final concentration of DMSO in culture media was adjusted to 0.1% (v/v). DMSO alone was run as a control in all cases.

**Chemicals and reagents**

Mouse anti-dinitrophenyl (DNP) IgE and DNP-human serum albumin (HSA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Fexofenadine-HCl (Fexo), a histamine H1 receptor antagonist, was obtained from Korea Pharma (Seoul, Korea). The rabbit polyclonal antibodies specific for phospho-IκB, IKKα/β, ERK1/2, JNK, p38, Akt, β-actin, and total form for IκB, ERK1/2, JNK, p38, and Akt were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Rabbit polyclonal antibodies for phospho-cPLAα (Ser505), cPLAα, 5-LO, PLCγ1, IKKα/β, lamin B and NF-κB p65 as well as secondary goat anti-rabbit IgG-HRP and rabbit anti-goat IgG-HRP antibodies, total Syk, total LAT, and Bay 61-3606 were purchased from Santa Cruz Biotechnology (Dallas, Texas, USA) and antibodies for phosphotyrosine was purchased from Millipore (Millipore, Billerica, MA, USA). The antibody reactive bands were visualized with an enhanced chemiluminescence (ECL) system (Pierce Biotechnology, Rockford, IL, USA). The enzyme immunoassay (EIA) kits for PGD2, LTC4, histamine and the antibody for COX-2 were purchased from Cayman Chemicals (Ann Arbor, MI, USA).

**Culture and activation of bone marrow derived mast cells (BMMCs)**

BMMCs were isolated from bone marrow of C57BL/6 mice and differentiated as described previously (Lu et al., 2011). Briefly, BMMCs were cultured in RPMI 1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin, 10 mM HEPES, 100 μM MEM non-essential amino acid solution (Invitrogen, Grand Island, NY, USA) and 20% pokeweed mitogen-spleen cell conditioned medium as a source of IL-3. For stimulation, 106 cells/ml were sensitized overnight with 500 ng/ml anti-DNP IgE, pretreated with indicated concentration of curcumin or Bay 61-3606, and stimulated for appropriate periods with 100 ng/ml DNP-HSA. The reactions were terminated by centrifugation of the cells at 3,000 rpm for 5 min at 4°C.

**Determination of LTC4 and PGD2**

Concentration of LTC4 and PGD2 were determined as described previously (Lu et al., 2011). IgE sensitized BMMCs suspended in enriched medium at a cell density of 1×10^6 cells/ml were pretreated with indicated concentration of curcumin or Bay 61-3606 for 1 h and stimulated with DNP-HSA for 15 min. Supernatants were isolated for further analysis by EIA kit. The concentration of LTC4 was determined using an EIA kit. To assess COX-2-dependent PGD2, synthesis, BMMCs were preincubated with 1 μg/ml of aspirin for 2 h to irreversibly inactivate preexisting COX-1. After washing, BMMCs were incubated with 100 ng/ml DNP-HSA at 37°C for 7 h in the presence of curcumin or Bay 61-3606. PGD2 in the supernatants were quantified using PGD2 EIA kit and cells were used for immunoblots analysis. Under the conditions employed, LTC4, reached 4.75 ng/10^6 cells and PGD2 generation reached 2.12 ng/10^6 cells. All data were the arithmetic mean of triplicate determinations.

**Measurement of intracellular Ca^{2+} level**

Intracellular Ca^{2+} levels were determined using FluoForte™ Calcium Assay Kit (Enzo Life Sciences, Ann Arbor, MI, USA), as described previously (Hwang et al., 2013). Briefly, BMMCs (1×10^6 cells) were sensitized overnight with 500 ng/ml anti-DNP IgE. Sensitized BMMCs were preincubated with FluoForte™ Dye-Loading Solution for 1 h at room temperature. After washing the dye from cell surface with HBSS, cells (5×10^5) were seeded into 96-well microplates and pretreated with curcumin or Bay 61-3606 for 1 h before adding DNP-HSA. Fluorescence was measured using a fluorometric imaging plate reader at an excitation wavelength of 485 nm and an emission wavelength of 520 nm on a BMG Labtechnologies FLUOSTar OPTITIMA platereader (Offenburg, Germany).

**Preparation of nuclear and cytoplasmic extracts**

The nuclear and cytoplasmic extracts were prepared as described previously (Lu et al., 2011). BMMCs were sensitized to DNP-specific IgE (500 ng/ml, overnight) and pretreated with curcumin or Bay 61-3606 for 1 h, and then stimulated with DNP-HSA (100 ng/ml) for 30 min. Cultured BMMCs were collected by centrifugation, washed with PBS, and lysed in a buffer containing 10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 1 mM PMSF and 0.1% NP40 on ice for 10 min. Supernatants (cytosolic fractions) were obtained by centrifugation at 1,000 g for 4 min. Nuclear pellets were washed and lysed in a buffer containing 20 mM HEPES (pH 7.9), 25% (v/v) glycerol, 420 mM NaCl, 1.5 mM...
The concentration of serum LTC₄ (A), PGD₂ (B), and histamine (C) were determined using appropriate enzyme immunoassay kits (Cayman Chemicals). Mice were injected with saline or injected with saline alone. 24 h later mice were administered 25 or 50 mg/kg of curcumin or 50 mg/kg fexofenadine-HCI (Fexo) and 1 h later were challenged with 4 mg i.v. of DNP-HSA in 200 μl saline; blood was collected by cardiac puncture 5 min after the Ag challenge. The concentration of serum LTC₄, PGD₂, and histamine (C) were determined using appropriate enzyme immunoassay kits (Cayman Chemicals). The values indicate the mean ± S.D. from three independent experiments, **p<0.01 versus IgE/Ag sensitized mice. Fexo (50 mg/kg) was used as an anti-histamine control drug.

Immunoprecipitation (IP)

Immunoprecipitation was performed as described previously (Hwang et al., 2013). Briefly, cell lysates were obtained using modified lysis buffer [0.1% Nonidet P-40, 50 mM HEPES (pH 7.0), 250 mM NaCl, 5 mM EDTA, 1 mM PMSF, and 0.5 mM dithiothreitol]. Total cell lysates (1 mg protein equivalent) were incubated with anti-Syk or anti-LAT antibodies for 2 h at 4°C. Precipitates and total cell lysates were subjected to SDS-PAGE and immunoblotted with appropriate antibodies.

Immunoblotting

Immunoblotting was performed as described previously (Lu et al., 2011). Cells were lysed in RIPA lysis buffer (50 mM Tris-HCl [pH 8.0], 150 mM NaCl, 5 mM EDTA, 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM dithiothreitol (DTT), 200 mM NaF, 200 mM Na₃VO₄, and protease inhibitor cocktail). Cell debris was removed by centrifugation at 14,000 g for 15 min at 4°C, and the resulting supernatant was used for 4°C and immunocomplexes were precipitated with 20 μl of protein A-Sepharose and washed 3 times with ice-cold lysis buffer. Precipitates and total cell lysates were subjected to SDS-PAGE and immunoblotted with appropriate antibodies.

RESULTS

Curcumin suppressed passive systemic anaphylaxis (PSA) in mice

Anaphylaxis is an IgE-mediated hypersensitivity reaction caused by the release of various inflammatory mediators due to binding of specific IgE to FcεRI on the surfaces of mast cells or basophils (Siraganian, 2003). Furthermore, it has been reported that curcumin suppresses IgE or compound 48/80-induced passive cutaneous anaphylaxis (Suzuki et al., 2005; Lee et al., 2008; Choi et al., 2010). However, no report has been issued on the effect of curcumin on PSA in mice. Therefore, we assessed the anti-allergic-inflammatory effects of curcumin using a mouse PSA model. As shown in Fig. 1, PSA was induced using an i.v. injection of DNP-HSA in ICR mice after the oral administration of 25 or 50 mg/kg of curcumin or 50 mg/kg of Fexo (Ciprandi et al., 2003). One hour later, curcumin reduced serum LTC₄ (Fig. 1A), PGD₂ (Fig. 1B) and histamine (Fig. 1C) levels in a dose-dependent manner (n=6). The suppressive effect of 50 mg/kg of curcumin was similar to that of 50 mg/kg of Fexo, a histamine H1 receptor antagonist.

Curcumin inhibited LTC₄ generation and Ca²⁺ influx in IgE/Ag-induced BMMCs

In vivo results let us to investigate the action mechanism responsible for the anti-allergic inflammatory activities of cur-
Fig. 2. Effect of Curcumin on LTC4 generation and on Ca2+ mobilization in IgE/Ag-activated BMMCs. IgE-sensitized BMMCs were pre-incubated with the indicated concentration of curcumin or Bay61-3606 for 1 h and then stimulated with DNP-HSA for 15 min. LTC4 released into the supernatant was quantified using an enzyme immunoassay kit (A). Relative intracellular Ca2+ levels were determined (at 5 min) (B). *p<0.05, **p<0.01 and ***p<0.001 versus the IgE/Ag-treated group. Results are the averages of three independent experiments.

Fig. 3. Effects of curcumin and Bay 61-3606 on cPLA2α and 5-LO translocation and MAPKs activation. IgE-sensitized BMMCs were pre-incubated for 1 h with the indicated concentrations of curcumin or Bay61-3606 and then stimulated with DNP-HSA for 15 min. Cytosolic and nuclear fractions were immunoblotted with antibodies for phospho-cPLA2α (Ser505) and 5-LO (A), and cell lysates were immunoblotted for the total and phosphorylated forms of ERK1/2, JNK and p38 (B). Immunoblots of β-actin and lamin B were used as controls for cytosol and nuclear fractions, respectively.

Fig. 4. Effect of Curcumin on COX-2 dependent PGD2 generation and on Akt-NF-κB activation. IgE-sensitized BMMCs were pre-incubated with the indicated concentration of curcumin or Bay61-3606 for 1 h and then stimulated with DNP-HSA for 7 h. PGD2 released into the supernatant was quantified using an enzyme immunoassay kit (A). Relative intracellular Ca2+ levels were determined (at 5 min) (B). *p<0.05, **p<0.01 and ***p<0.001 versus the IgE/Ag-treated group. Results are the averages of three independent experiments.

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Curcumin using mast cells, major players in PSA. LTC₄ is metabolites of arachidonic acid derived from action of 5-LO/LTC₄ synthase in mast cells, and LTC₄ have been implicated in inflammation, proliferation and allergic conditions like asthma (Murphy and Gijon, 2007). Thus, we investigated the effects of curcumin on 5-LO dependent LTC₄ generation in BMCCs. As shown in Fig. 2A, curcumin strongly inhibited LTC₄ generation in a dose-dependent manner. It is well known that Ca²⁺ is essential for arachidonic acid (AA) release from phospholipid and degranulation in IgE/Ag-induced mast cells (Kudo and Murakami, 1999; Yamaguchi et al., 1999), thus we examined the effect of curcumin on intracellular Ca²⁺ influx in IgE/Ag-induced BMCCs. As shown in Fig. 2B, intracellular Ca²⁺ level in activated BMCCs was about three fold higher than in resting cells, and this increase was dose-dependently inhibited by curcumin. Bay 61-3606 (a Syk inhibitor) also strongly decreased intracellular LTC₄ generation as well as Ca²⁺ influx. Consistent with a previous report (Lee et al., 2008), the release of β-Hex (a degranulation marker enzyme) was dose-dependently inhibited by curcumin (data not shown).

Curcumin inhibited cPLA₂ phosphorylation, translocation of phospho-cPLA₂α and 5-LO and activation of MAPKs

Recently, we and other group have reported that the release of free AA from membrane phospholipid in activated mast cells requires the phosphorylation of cPLA₂α (p-cPLA₂α) by mitogen activated protein kinases (MAPKs) (Lin et al., 1993; Lu et al., 2011), and the translocation of p-cPLA₂α is dependent on intracellular Ca²⁺ influx (Gijon and Leslie, 1999; Lu et al., 2011). To determine whether curcumin inhibits the phosphorylation and translocation of cPLA₂α, we pretreated BMCCs with different concentrations of curcumin or Bay 61-3606. As show in Fig. 3A, curcumin dose-dependently inhibited the phosphorylation (C-p-cPLA₂α) and translocation of C-p-cPLA₂α to the nuclear envelope (N-p-cPLA₂α). The synthesis of 5-LO dependent LTC₄ in IgE/Ag-induced mast cells is known to mediate the translocations of both p-cPLA₂α and 5-LO to the nuclear envelope (Werz, 2002; Lu et al., 2011; Lu et al., 2012). Thus, we investigated the effect of curcumin on the translocation of 5-LO to the nuclear envelope. As was expected, curcumin or Bay 61-3606 inhibited the translocation of cytosolic 5-LO (C-5-LO) to nuclear envelope (N-5-LO); Next, to confirm that the inhibition of cPLA₂α phosphorylation by curcumin occurred via the inhibition of MAPKs phosphorylation including extracellular regulated kinase1/2 (ERK1/2), c-jun N-terminal kinase (JNK), and p38 MAP kinase, therefore we examined the effect of curcumin or Bay 61-3606 on the phosphorylation of MAPKs. As shown in Fig. 3B, curcumin or Bay 61-3606 inhibited the phosphorylations of three MAPKs in a dose dependent manner.

Curcumin inhibits COX-2 dependent PGD₂ generation and NF-κB activation

In mast cells, unlike 5-LO dependent LTC₄ generation, PGD₂ generation occurs in a biphasic manner. Immediate PGD₂ generation (occurring within 2 h), is associated with COX-1, and delayed PGD₂ generation (during 2-10 h) is occurred by inducible COX-2 protein (Ashraf et al., 1996; Moon et al., 1998). To assess COX-2-dependent delayed PGD₂ generation, BMCCs were pre-treated with aspirin to abolish preexisting COX-1 activity, followed by a brief wash, and then stimulated with Ag for 7 h with or without curcumin. As shown in Fig 4A, delayed

Fig. 5. Effect of curcumin on the Syk pathway. IgE-sensitized BMCCs were preincubated with curcumin or Bay 61-3606 for 1 h, and then stimulated with DNP-HSA for 5 min. Cell lysates were subjected to immunoprecipitation and immunoblot analysis for the phosphorylated forms of Syk, LAT and PLCγ1. Bay 61-3606 was used as a positive control with respect to the suppression of the Syk-mediated pathway. The relative ratios of p-Syk/Syk, p-LAT/LAT and p-PLCγ1/PLCγ1 protein levels were determined by measuring immunoblot band intensities by scanning densitometry (**p<0.01 and ***p<0.001). The results shown are representative of three independent experiments.
PGD$_2$ generation was dose-dependently inhibited by curcumin, with a concomitant suppression of COX-2 protein expression. Yu et al., reported that the Syk downstream molecules PI3K/Akt pathway affects transcription factor NF-$\kappa$B activation in gastric cancer cells and mast cells (Yu et al., 2010; Lu et al., 2011) and NF-$\kappa$B has been identified as an essential transcription factor for the induction of several inflammatory mediators including, TNF-$\alpha$, COX-2, and inducible NO synthase (Reddy et al., 2000; Tak and Firestein, 2000; Lu et al., 2011). Thus, we examined the effect of curcumin on Akt/NF-$\kappa$B axis activation. When IgE-sensitized BMMCs were pretreated with curcumin for 1 h and then stimulated with Ag for 15 min, phosphorylation of the Akt, IKK complex (p-IKK$\alpha$/$\beta$) and I$\kappa$B$\alpha$ (p-I$\kappa$B$\alpha$) was increased, with a concomitant decrease of total I$\kappa$B$\alpha$ and nuclear translocation of NF-$\kappa$B (C-NF-$\kappa$B). As shown in Fig. 4B, both curcumin and Bay 61-3606 inhibited the phosphorylations of Akt, IKK$\alpha$/$\beta$, I$\kappa$B$\alpha$, I$\kappa$B$\alpha$ degradation and the translocation of cytosolic p65 to nucleus (N-NF-$\kappa$B), suggesting that Syk mediated Akt/NF-$\kappa$B pathway regulate the reduction of COX-2 dependent PGD$_2$ by curcumin.

**Curcumin inhibited the Syk pathway in IgE/Ag-induced BMMCs**

Previously, we and others reported that Syk plays an essential role in the initiation of Fc$\gamma$RI-induced mast cells activation and mediates LAT, resulting in the activation of downstream signaling, including MAPKs, phosphatidylinositol 3-kinase (PI3K), and PLC$_\gamma$ (Lin et al., 1993; Lu et al., 2011). Furthermore, it has been reported that curcumin inhibited the releases of TNF-$\alpha$ and IL-4 from mast cells via a Syk dependent pathway, and that the inhibitions of these secretion by curcumin is dependent on its direct inhibition of Syk kinase activity rather than Syk phosphorylation (Lee et al., 2008). To determine whether curcumin affects Syk phosphorylation in IgE/Ag-induced BMMCs, we examined the effects of curcumin on the phosphorylation of Syk and its downstream signal molecules LAT and PLC$_\gamma$1. As shown in Fig. 5A, curcumin did not affect Syk phosphorylation, but significantly and dose-dependently inhibited the phosphorylations of LAT and PLC$_\gamma$1 (Fig. 5B, C), as previously reported (Lee et al., 2008). Bay 61-3606 (the Syk inhibitor used as a positive control) completely inhibited the phosphorylations of Syk, LAT and PLC$_\gamma$1. These results suggest that curcumin inhibits PGD$_2$, LTC$_4$, and degranulation by regulating the Syk signal pathway.

**DISCUSSION**

Curcumin has been shown to have diverse biological activities, such as, antioxidant, anti-allergic, anti-inflammatory, antiviral, antibacterial, antifungal, and anticancer activities (Ammon and Wahl, 1991). Several groups have reported that curcumin shows anti-allergic activity through the inhibition of histamine release, TNF-$\alpha$ and IL-4 from activated mast cells and in vivo type I hypersensitivity animal model (Yano et al., 2000; Ram et al., 2003; Lee et al., 2008). In addition, curcumin also suppresses arachidonic acid metabolizing enzymes such as cPLA$_2$ phospholipase, COX-2 expression and recombinant 5-LO activity in LPS-stimulated RAW 264.7 cells and A23187-stimulated HT human colon cancer cells (Hong et al., 2004) and inhibits in vitro LOX and COX activities in TPA- and arachidonic acid-induced inflammation in mouse epidermis (Huang et al., 1991). Furthermore, Curcumin modulates the inflammatory response by down-regulating the activity of COX-2 and inducible nitric oxide synthase (iNOS) enzymes through suppression of NF-$\kappa$B activation (Suru et al., 2001). Even though they reported that curcumin inhibited generation of COX-dependent PGs and LOX-dependent hydroxyeicosatetraenoic acids (HETEs), previous reports were mainly examined as a part of anticancer activity of curcumin. However, the effect of curcumin on generation of COX-2 dependent PGD$_2$ and 5-LO dependent LTC$_4$ generation in IgE/Ag-induced mast cells and IgE-mediated PSA reaction have not been studied to date. Therefore, we investigated the effects of curcumin on the generation of eicosanoid (PGD$_2$ and LTC$_4$) in BMMCs and on PSA reaction in mice. It has been well established that lipid mediators, like LTC$_4$ and PGD$_2$, are closely associated with various allergic and inflammatory diseases (Werz and Steinhalber, 2006; Ricciotti and FitzGerald, 2011). Thus, the inhibition of LTC$_4$ and PGD$_2$ generation by mast cells is an important therapeutic strategy in the context of allergic-inflammatory disease. The aim of this study was to investigate the effect of curcumin on IgE/Ag-induced COX-dependent PGD$_2$ and 5-LO dependent LTC$_4$ generation in BMMCs and on IgE-induced passive systemic anaphylaxis (PSA) in mice. IgE binds to Fc$\gamma$RI on mast cells, the Syk-LAT axis activates PLC$_\gamma$, which increases intracellular Ca$^{2+}$ influx and leads to degranulation and eicosanoid production (Siragangian, 2003). As described above, IgE binds to Fc$\gamma$RI on BMMCs, it promptly elicits 5-LO dependent LTC$_4$ generation, which is inhibited dose-dependently by curcumin (Fig. 2A). It has been reported that the synthesis of LTC$_4$ in mast cells is regulated by two steps, namely, AA release from phospholipid by cPLA$_2$, MAPKs-mediated phosphorylation of cPLA$_2$, and the conversion of free AA to LTC$_4$ by 5-LO (Fischer et al., 2005). Translocation of both 5-LO and phospho-cPLA$_2$ (C-p-cPLA$_2$) to the nuclear envelope are depend on the increase of cytosolic Ca$^{2+}$ level (Werz, 2002; Lu et al., 2011; Lu et al., 2012). The present study showed that curcumin inhibited the translocations of both enzymes, which concurred with its observed inhibitory effect on intracellular Ca$^{2+}$ influx (Fig. 2B). Next, to elucidate the effect of curcumin on COX-2 dependent delayed PGD$_2$ generation, BMMCs were pre-treated with aspirin to abolish preexisting COX-1 activity, and then stimulated with Ag for 7 h with or without curcumin. We found that curcumin also suppressed COX-2 expression and attendant PGD$_2$ generation (Fig. 4A). It has been previously reported that curcumin inhibited the TPA-induced up-regulation of COX-2 and MMP-9 by suppressing ERK1/2 phosphorylation and NF-$\kappa$B transactivation in epithelial cells (Lee et al., 2005), and that it exerts anti-inflammatory effects by inhibiting the NF-$\kappa$B and MAPKs pathways (Cho et al., 2007). Recently, we also reported that COX-2-dependent PGD$_2$ generation and COX-2 expression in BMMCs occurs via the activation of the NF-$\kappa$B and MAPKs pathways (Lu et al., 2011; Lu et al., 2012), thus we investigated the effect of curcumin on the MAPKs and NF-$\kappa$B pathways in IgE/Ag-induced BMMCs. As shown in Fig. 3B, curcumin suppressed the phosphorylations of ERK1/2, JNK, and p38 MAP kinase in a dose dependent manner, which implied that it affects the phosphorylation and translocation of cPLA$_2$. Since curcumin or Bay 61-3606 suppressed intracellular Ca$^{2+}$ influx (Fig. 2B), MAPKs phosphorylation (Fig. 3B) and Akt/ NF-$\kappa$B axis activation, these results suggest that Syk plays an important role in the generation of PGD$_2$ and LTC$_4$ in IgE/Ag-induced BMMCs. Therefore, we examined whether curcumin affects the phos-
phorylation of Syk in IgE/Ag-induced BMMCs. In agreement with a previous report (Lee et al., 2008), curcumin was found to not directly inhibit the phosphorylation of Syk, but to inhibit the phosphorylations of LAT and PLCγ1 which lie downstream of Syk. In view of the effect of phosphorylated PLCγ1 on inositol phospholipid turnover and the consequent increase in Ca2+ influx, it is likely that the observed inhibition of Ca2+ influx by curcumin (Fig. 2B) depends on its inhibitory effect on Syk-dependent PLCγ1 phosphorylation. In addition, to our in vitro results, curcumin also suppressed the IgE-dependent PSA reaction in a mast cell-dependent in vivo model of systemic allergic reaction (Wershil et al., 1987) with a potency equivalent to that of the H1 histamine antagonist, Fexofenadine (Fig. 1A-C). We already reported that PGD2 and LTC4 play important roles in mast cell-mediated anaphylactic reaction (Lu et al., 2011; Hwang et al., 2013). Recently, several reports have demonstrated that Syk kinase inhibitors promised for the treatment of allergic and antibody-mediated autoimmune diseases (Ruzza et al., 2009) and clinical implications of Syk inhibitor showed antiallergic properties when administered orally (Mazuc et al., 2008). Taken together with previous results (Lee et al., 2008), the anti-allergic inflammatory activity of curcumin appear to be due to the suppressions of the secretions of TNF-α and IL-4 and histamine release and eicosanoid generation through the inhibition of Syk kinase pathway in IgE/Ag-induced mast cells.

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