

Curcumin, a constituent of curry, suppresses IgE-mediated allergic response and mast cell activation at the level of Syk

Jun Ho Lee, PhD,^a Jie Wan Kim, MSc,^a Na Young Ko, MSc,^a Se Hwan Mun, MSc,^a Erk Her, PhD,^a Bo Kyung Kim, PhD,^a Jeung Whan Han, PhD,^b Hoi Young Lee, PhD,^c Michael A. Beaven, PhD,^d Young Mi Kim, PhD,^e and Wahn Soo Choi, PhD^a Chungju, Suwon, Nonsan, and Seoul, Korea, and Bethesda, Md

Background: Activation of mast cells through the high-affinity receptor for IgE (FcεRI) underlies atopic allergic reactions. Curcumin can block this activation, but the mechanism and the effects of curcumin on IgE-mediated allergic reactions are unknown.

Objectives: We sought to determine the antiallergic activity of curcumin *in vivo* and its mechanism of action in mast cells.

Methods: The antiallergic activity of curcumin was evaluated in mast cell cultures and the passive cutaneous anaphylaxis model. The effects of curcumin on mast cell signaling events were examined by using immunoblotting, immunoprecipitation, RT-PCR, and other molecular biologic approaches.

Results: Curcumin inhibited antigen-mediated activation of mast cells and passive cutaneous anaphylaxis in mice.

Suppression of degranulation and secretion of TNF-α and IL-4 was apparent at concentrations as low as 3 μmol/L curcumin in activated mast cells. Similar concentrations of curcumin suppressed Syk-dependent phosphorylations of the adaptor proteins linker of activated T cells and Grb2-associated binder 2, which are critical for mast cell activation. Although curcumin did not inhibit the phosphorylation of Syk itself, it directly inhibited Syk kinase activity *in vitro*. Further downstream, activating phosphorylations of Akt and the mitogen-activated protein kinases p38, p44/42 (extracellular signal-regulated

kinase 1/2), and c-Jun N-terminal kinase, which are critical for the production of inflammatory cytokines, were also inhibited.

Conclusions: Curcumin inhibits Syk kinase-dependent signaling events in mast cells and might thus contribute to its antiallergic activity. Therefore curcumin might be useful for the treatment of mast cell-related immediate and delayed allergic diseases. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Curry spice, curcumin, allergy, passive cutaneous anaphylaxis, mast cells, IgE, Syk kinase

Curcumin, which imparts the yellow color to curry, is a natural product of the spice turmeric, *Curcuma longa* L (Zingiberaceae). Curcumin exhibits a variety of pharmacologic activities, including anti-inflammatory, anticancer, antioxidant, wound-healing, and antimicrobial effects.¹ Curcumin also possesses antiallergic activity when examined in animal models of allergy,^{2,3} and it inhibits degranulation of the RBL-2H3 tumor mast cell line in culture.^{4,5} However, the mechanisms responsible for its antiallergic activity at the cellular and molecular level are unknown.

Approaches to the treatment of allergic diseases include allergen-specific immunotherapy, DNA vaccination, humanized anti-IgE antibody administration, soluble IL-4 receptor treatment, use of antagonists of leukotriene and histamine receptors, and treatment with glucocorticoids.^{6,7} Another approach under consideration is the use of inhibitors of tyrosine kinases, such as Syk, that provide specific therapeutic targets for suppression of mast cell activation.⁸ It is well established that mast cells are associated with various allergic and autoimmune disorders.^{9,10} These cells respond to IgE-mediated antigens through the high-affinity receptor for IgE, FcεRI, by release of granules that contain preformed inflammatory mediators and the generation of inflammatory lipids and cytokines.¹¹

Syk kinase, which is the cognate of the T-cell kinase ZAP70 in mast cells, plays an essential role in the IgE-dependent activation of mast cells. Syk kinase is initially activated through phosphorylation by FcεRI-associated Src-family tyrosine kinases, primarily Lyn.¹² Syk, in turn, phosphorylates tyrosines on the adaptor proteins linker of activated T cells (LAT) and Grb2-associated binder 2 (Gab2) to provide docking sites for assembly of other signaling molecules to ensure further propagation of activating signals. Such signals include the phosphorylation and activation of phospholipase Cγ and the mobilization of calcium to generate a critical signal for degranulation.¹³ Other downstream signals, such as the activation of phosphatidylinositol 3-kinase¹⁴ and mitogen-activated protein (MAP) kinases,^{15,16} promote the activation of

From ^athe Department of Immunology and physiology, College of Medicine, Konkuk University, Chungju; ^bthe College of Pharmacy, Sungkyunkwan University, Suwon; ^cthe College of Medicine, Konyang University, Nonsan; ^dthe Laboratory of Immunology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda; and ^ethe College of Pharmacy, Duksung Women's University, Seoul.

Supported by a Korea Research Foundation grant funded by the Korea Government (MOEHRD; the Regional Research Universities Program/Chungbuk BIT Research-Oriented University Consortium); by the Regional Innovation Center Program of the Ministry of Commerce, Industry, and Energy; and in part by the 2nd phase BK21 program of the Ministry of Education and Human Resources Development in Korea. Michael A. Beaven was supported from the Intramural Program of the National Institutes of Health.

Disclosure of potential conflict of interest: M. A. Beaven has received research support from the National Heart, Lung, and Blood Institute and the National Institutes of Health. The rest of the authors have received research support from the Korea Research Foundation; the Regional Innovation Center Program of the Ministry of Commerce, Industry, and Energy; and the 2nd phase BK21 program of the Ministry of Education and Human Resources Development in Korea.

Received for publication September 22, 2007; revised December 14, 2007; accepted for publication December 18, 2007.

Reprint requests: Wahn Soo Choi, PhD, Department of Immunology, College of Medicine, Konkuk University, Chungju 380-701, Korea. E-mail: wahnchoi@kku.ac.kr.

0091-6749

doi:10.1016/j.jaci.2007.12.1160

Abbreviations used

BMMC: Bone marrow–derived mast cell
 DNP: Dinitrophenol
 DPH: Diphenylhydramine
 Gab2: Grb2-associated binder 2
 IC₅₀: Inhibitory concentration of 50%
 LAT: Linker of activated T cells
 MAP: Mitogen-activated protein
 PCA: Passive cutaneous anaphylaxis
 PIPES: Piperazine-N-N'-bis(1-ethanesulfonic acid)

transcription factors for the production of cytokines, including TNF- α and IL-4. Therefore blockade of Syk activity would result in suppression of release of multiple inflammatory mediators and might have broad beneficial effects because each of these mediators plays distinct but overlapping roles in the inflammatory response.¹⁷

In this study we describe the antiallergic activity of curcumin and its mechanism of action in mast cells. We show that curcumin reversibly suppresses degranulation and secretion of inflammatory cytokines, such as TNF- α and IL-4, through inhibition of Syk kinase and Syk kinase–dependent signaling events in mast cells.

METHODS

Reagents

Reagents were from the following sources: PP2 from Calbiochem (La Jolla, Calif); antibodies against the phosphorylated forms of extracellular signal-regulated kinase 1/2 (monoclonal), p38 (polyclonal), c-Jun N-terminal kinase (polyclonal), Akt (polyclonal), and Syk (Y352 in human subjects) from Cell Signaling Technology, Inc (Danvers, Mass); antibodies against Syk (polyclonal) and actin (monoclonal) from Santa Cruz Biotechnology, Inc (Santa Cruz, Calif); antibody (4G10) against phosphotyrosine and antibodies against LAT and Gab2 from Upstate Biotechnology (Lake Placid, NY); cell-culture reagents from GIBCO/Life Technologies, Inc (Rockville, Md); and curcumin, dinitrophenol (DNP)–specific monoclonal IgE, DNP-BSA, and other reagents from Sigma-Aldrich Corp (St Louis, Mo).

Animals

Male BALB/c mice (aged 4 weeks) were purchased from the Dae Han Experimental Animal Center (Eumsung, Korea) and were housed in the animal facilities at the College of Medicine of Konkuk University. Ten mice were placed in each cage within a laminar airflow cabinet. The mice were kept at a temperature of 22°C $\pm$ 1°C and at a relative humidity of 55% $\pm$ 10% throughout the study. This study was done in accordance with the institutional guidelines.

Induction of mast cell–mediated passive cutaneous anaphylaxis in mice

An anti-DNP-BSA–specific IgE (0.5 μ g) was intradermally injected into a mouse ear, followed 24 hours later by oral administration of curcumin in doses of 0.5 to 50 mg/kg or 50 mg/kg diphenylhydramine (DPH) as a control. One hour later, the mice were then challenged with an intravenous injection of a 250- μ g antigen (DNP-BSA) in 250 μ L of PBS containing 4% Evans blue. The mice were then killed an hour after the antigen challenge, followed by the removal of an ear for measurement of the amount of dye extravasated by the antigen. The dye was extracted overnight from the ear in 700 μ L of formamide at 63°C. The intensity of the absorbance was measured at 620 nm.

Preparation and stimulation of bone marrow–derived mast cells and RBL-2H3 cells

Bone marrow–derived mast cells (BMMCs) from male BALB/c mice were cultured for up to 10 weeks in a 50% enriched medium (RPMI 1640, containing 2 mM L-glutamine, 0.1 mM nonessential amino acids, antibiotics, and 10% FCS) containing 10 ng/mL IL-3. After 3 weeks, the cells were used for the study after verifying that cells expressed the Fc ϵ R1 β subunit and degranulated in response to IgE/antigen. For cell stimulation, BMMCs were primed overnight with 50 ng/mL DNP-specific IgE. The BMMCs were washed and resuspended in a Tyrode-BSA buffer (20 mM HEPES [pH 7.4], 135 mM NaCl, 5 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5.6 mM glucose, and 0.05% BSA) before stimulation with 20 ng/mL DNP-BSA for 10 minutes. RBL-2H3 cells were grown as monolayers in minimal essential medium with Earle's salts, supplemented with glutamine, antibiotics, and 15% FBS.

Measurement of degranulation in BMMCs and RBL-2H3 mast cells

In each experiment cells were transferred to 24-well (2 $\times$ 10⁵ cells per 0.4 mL per well) cluster plates and were incubated overnight in a complete growth medium with 50 ng/mL DNP-specific IgE. The cultures were washed, and the required buffered solution was added (0.2 mL per well). Experiments on intact cells were performed in a piperazine-N-N'-bis(1-ethanesulfonic acid) (PIPES)–buffered medium (25 mmol/L PIPES [pH 7.2], 159 mmol/L NaCl, 5 mmol/L KCl, 0.4 mmol/L MgCl₂, 1 mmol/L CaCl₂, 5.6 mmol/L glucose, and 0.1% fatty acid–free fraction V from a bovine serum) for RBL-2H3 cells and in a Tyrode buffer for BMMCs. Unless stated otherwise, the cultures were incubated for 30 minutes with or without curcumin before adding 25 ng/mL DNP-BSA for 10 minutes. The secretion of granules containing various allergic mediators was determined by use of a colorimetric assay to measure release of the granule marker β -hexosaminidase.¹⁸ For this assay, release of *p*-nitrophenol from *p*-nitrophenyl-N-acetyl- β -D-glucosaminide was measured. The values were expressed as percentages of intracellular β -hexosaminidase released into the medium. The inhibitory concentration of 50% (IC₅₀) was calculated through nonlinear regression analysis by using the GraphPad software (San Diego, Calif).

Immunoblotting analysis

BMMCs were washed, and the medium was replaced with the Tyrode buffer. The cells were then stimulated with 25 ng/mL of antigen (DNP-BSA) for 10 minutes with or without curcumin and then chilled with ice to terminate the stimulation. Thereafter, the cells were washed twice with ice-cold PBS and lysed in 0.5 mL with an ice-cold lysis buffer (20 mmol/L HEPES [pH 7.5], 150 mmol/L NaCl, 1% Nonidet P-40, 10% glycerol, 60 mmol/L octyl β -glucoside, 10 mmol/L NaF, 1 mmol/L Na₃VO₄, 1 mmol/L phenylmethylsulfonyl fluoride, 2.5 mmol/L nitrophenylphosphate, 0.7 μ g/mL pepstatin, and a protease inhibitor cocktail tablet). The lysates were kept on ice for 30 minutes, followed by centrifugation at 15,000g for 15 minutes at 4°C. The cell lysates were denatured by boiling at 95°C for 5 minutes in a 2 $\times$ Laemmli buffer.¹⁹ The proteins were separated with SDS-PAGE and were then transferred to nitrocellulose membranes (Schleicher and Schuell, BA85, Keene, NH). Subsequent to blocking in a TBS-T buffer (10 mmol/L Tris-HCl [pH 7.5], 150 mmol/L NaCl, and 0.05% Tween 20) containing 5% skimmed milk powder, the membrane was incubated with individual antibodies. The primary antibodies were diluted 1:2000-fold unless otherwise noted and were incubated at 4°C overnight. The membranes were washed 3 times for 5 minutes each with a TBS-T buffer. The immunoreactive proteins were incubated with the use of horseradish peroxidase–coupled secondary antibodies diluted 1:2000-fold for 1 hour at room temperature, subsequently washed 5 times (5 minutes each wash) with a TBS-T buffer, and developed with enhanced chemoluminescence, according to the manufacturer's protocols (Amersham Biosciences, Piscataway, NJ).

RT-PCR for TNF- α and IL-4 mRNA

The RBL-2H3 cells were harvested by means of trypsinization and were transferred to 6-well (1×10^6 cells per 3 mL per well) cluster plates, which were incubated overnight in a complete growth medium containing 25 ng/mL DNP-specific IgE. The cells were washed, and medium was replaced with a PIPES-buffered medium before stimulation with 25 ng/mL DNP-BSA for 15 minutes with or without curcumin. Cultures were then washed twice with ice-cold PBS. Total RNA was isolated by using Trizol Reagent (Invitrogen, Carlsbad, Calif) and was reverse transcribed with the Superscript first-strand synthesis system (Invitrogen), according to the manufacturer's protocol. PCR was performed at 94°C for 45 seconds, at 55°C for 45 seconds, and at 72°C for 60 seconds for 30 cycles. The following primers were used: rat TNF- α forward 5'-CACCACGCTCTTCTGTCTACTGAAC-3'; rat TNF- α reverse 5'-CCGGACTCC GTGATGTCTAAGTACT-3'; rat IL-4 forward 5'-ACCTTGCTGTCAACCTGTTC-3'; rat IL-4 reverse 5'-TTGTGAGCGTG GACTCATTC-3'; rat glyceraldehyde-3-phosphate dehydrogenase forward 5'-GTGGAGTCTACTGGC GTCTTC-3'; and rat glyceraldehyde-3-phosphate dehydrogenase reverse 5'-CCAAGGCTGTGGGCAAGGTCA-3'.

ELISA of TNF- α and IL-4

The RBL-2H3 cells were stimulated with 25 ng/mL DNP-BSA for 4 hours with or without curcumin. TNF- α and IL-4 concentrations in the culture supernatant were determined by using commercial ELISA kits, according to the manufacturer's instructions (Invitrogen-Biosource Cytokine & Signaling, Camarillo, Calif).

In vitro Syk kinase assay

The final reaction mixture (25 μ L) consisted of 10 mU Syk kinase (Millipore Corp, Billerica, Mass), 50 mmol/L Tris (pH 7.5), 0.1 mmol/L ethyleneglycol-bis-(β -aminoethylether)-N,N,N',N'-tetraacetic acid, 0.1 mM Na₃VO₄, 0.1% β -mercaptoethanol, 0.1 mg/mL poly (Glu, Tyr) 4:1, 10 mmol/L Mg acetate, and [γ ³³P]-ATP. The reaction was initiated by the addition of the Mg acetate and [γ ³³P]-ATP. After incubation for 40 minutes at room temperature, the reaction was stopped by the addition of 5 μ L of a 3% phosphoric acid solution. Ten microliters of the reaction mixture was then spotted onto a Filtermat A (Millipore Corp) and washed 3 times for 5 minutes in 75 mM phosphoric acid and once in methanol before drying and scintillation counting.

Statistical analysis

The data were presented as the means $\pm$ SEMs from 3 or more separate experiments. Statistical analysis was performed by using 1-way ANOVA and the Dunnett test. All statistical calculations (* P < .05 and ** P < .01) were performed with SigmaStat software (Systat Software, Inc, Point Richmond, Calif).

RESULTS

Curcumin suppresses the mast cell-mediated passive cutaneous anaphylaxis reaction in mice

We first examined the antiallergic activity of curcumin by use of the passive cutaneous anaphylaxis (PCA) in the mouse ear model.²⁰ PCA was successfully induced through the local injection of IgE in the mouse ear and then systemic injection of antigen in mice. Oral administration of curcumin 1 hour before injection of antigen significantly suppressed the mast cell-dependent PCA reaction in a dose-dependent manner, resulting in a median effective concentration of approximately 2.0 mg/kg (Fig 1). Nearly complete suppression of the PCA reaction was achieved with 50 mg/kg curcumin, as was true for the antihistamine DPH at the same dose (Fig 1).

Curcumin reversibly inhibits antigen-induced degranulation of mast cells

In view of the inhibitory effects of curcumin on PCA *in vivo*, we next examined its effects on RBL-2H3 cells, a widely used transformed mast cell line, and primary cultures of BMMCs to determine its mechanism of action in mast cells. BMMCs were found to express the Fc ϵ RI β subunit (data not shown) and degranulated in response to antigen stimulation after priming with the antigen-specific IgE (Fig 2, A). Curcumin significantly suppressed antigen-induced degranulation in a dose-dependent manner in both RBL-2H3 cells and BMMCs (Fig 2, A). The IC₅₀ values were 6.1 μ mol/L and 5.2 μ mol/L, respectively. This effect of curcumin was found to be reversible. The RBL-2H3 cells were washed out 5 times with PIPES buffer after incubating with curcumin for 1 hour to achieve the maximal inhibition of curcumin and then stimulated with antigen. As shown in Fig 2, B, degranulation of mast cells was restored after removal of curcumin.

Curcumin inhibits secretion and expression of TNF- α and IL-4 in mast cells

Various cytokines, including TNF- α and IL-4, are critical for allergic inflammation.²¹ Accordingly, we examined whether curcumin suppressed the expression and secretion of TNF- α and IL-4 in antigen-stimulated RBL-2H3 cells. Curcumin significantly inhibited the antigen-stimulated expression of mRNA for both cytokines in a dose-dependent manner (Fig 3, A). To further prove the above results, we tested whether curcumin suppressed secretion of TNF- α and IL-4 in the cells. In agreement with the RT-PCR results, curcumin inhibited secretion of both cytokines in a dose-dependent manner (Fig 3, B).

Curcumin inhibits Syk-dependent signaling events in antigen-stimulated cells

To gain insight as to how curcumin suppresses mast cell activation, we examined its effects on early Fc ϵ RI-mediated signaling events, namely the activating phosphorylation of Syk by Lyn and the phosphorylation of LAT and Gab2 by Syk. As shown in Fig 4, A, the tyrosine phosphorylation of Syk was not inhibited by curcumin in antigen-stimulated RBL-2H3 cells. Also, the phosphorylation of Syk tyrosine residue at 317 in mouse BMMCs (equivalent to Y352 in human subjects), which is the known site of phosphorylation by Lyn in mast cells,^{22,23} was not inhibited by curcumin (Fig 4, A). These results indicated that activation of upstream Src kinases and the phosphorylation of Syk itself were unaffected by curcumin. However, the Syk-dependent downstream activating phosphorylations of LAT and Gab2 were significantly inhibited by curcumin in a dose-dependent manner in both RBL-2H3 cells and BMMCs (Fig 4, A). The effects of curcumin on recombinant Syk kinase was examined by use of an *in vitro* assay system to verify that Syk kinase is a direct target of curcumin. As shown in Fig 4, B, Syk kinase was inhibited by curcumin, with an IC₅₀ of approximately 10 μ mol/L. The inhibitory effects of curcumin were apparent with as little as 3 μ mol/L, as was true for the suppression of degranulation (Fig 2), cytokine expression and secretion (Fig 3), and phosphorylation of LAT and Gab2 (Fig 4, A).

As noted, the phosphorylation and activation of phospholipase C γ , as well as calcium mobilization, are dependent on the

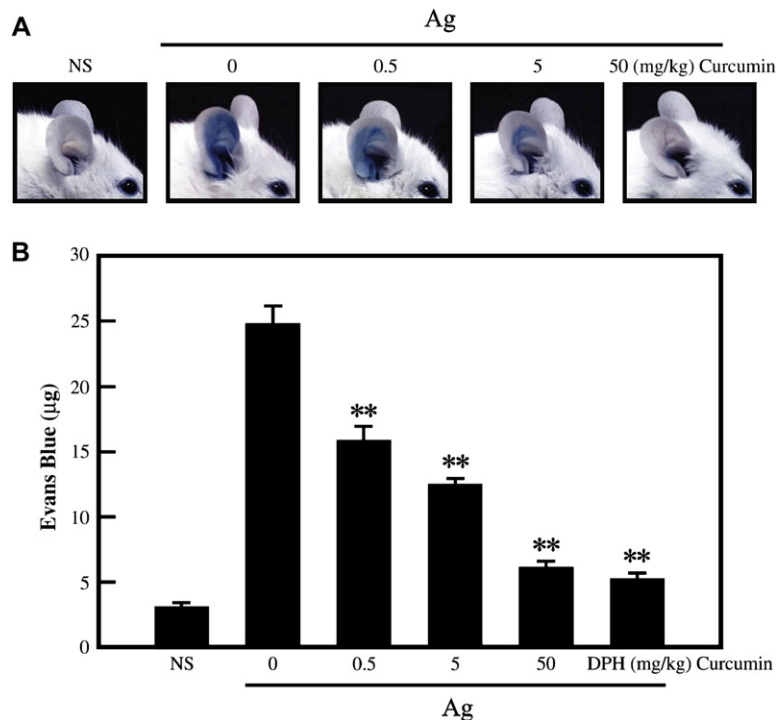


FIG 1. Curcumin inhibits the mast cell-mediated PCA reaction. **A**, Each picture shown is representative of 3 independent experiments. **B**, The means $\pm$ SEMs of values from 3 independent experiments, each with 10 mice, are shown. The asterisks indicate a significant difference from controls (no curcumin) at a *P* value of less than .001. DPH (50 mg/kg) was used as a typical antihistamine control drug.

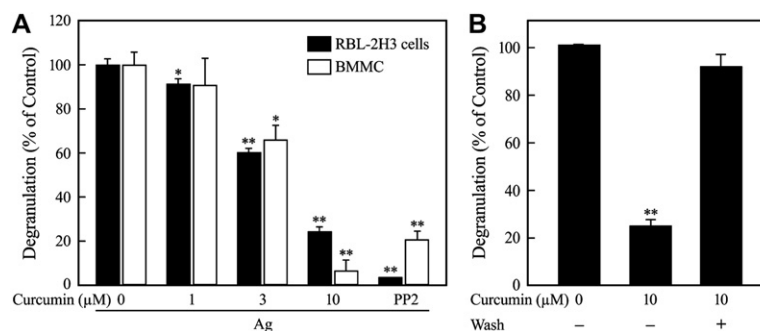


FIG 2. Curcumin reversibly inhibits antigen-induced degranulation in mast cells. **A**, Release of β -hexosaminidase was measured in antigen-stimulated BMMCs and RBL-2H3 cells. **B**, Reversibility of curcumin in RBL-2H3 cells. The values indicate the means $\pm$ SEMs from 3 independent experiments. Significant differences with controls (no curcumin) are indicated as follows: **P* < .05 and ***P* < .01. PP2 (10 μmol/L) is a general Src-family kinase inhibitor.

phosphorylation of Syk and LAT.¹³ Indeed, the phosphorylation of phospholipase C γ 1 was inhibited by curcumin in a concentration-dependent manner, with an IC₅₀ of approximately 2 μmol/L (Fig 5, A). However, we were unable to determine whether curcumin had corresponding inhibitory effects on calcium mobilization because the light-absorption and fluorescent properties of curcumin interfered with the measurement of cytosolic Ca²⁺ when tested with a variety of fluorescent Ca²⁺ probes.

We also examined the effects of curcumin on phosphatidylinositol 3-kinase¹⁴ and the MAP kinases^{15,16} because of their role in the production of TNF- α and IL-4. The phosphorylation of Akt, a

surrogate for the activation of phosphatidylinositol 3-kinase, and the activating phosphorylation of the MAP kinases p38, Erk1/2, and c-Jun N-terminal kinase were also significantly suppressed by curcumin in a dose-dependent manner. The IC₅₀ values for curcumin against the tested signaling molecules in Fig 5, B, were less than 3 μmol/L.

DISCUSSION

Mast cells participate in a variety of allergic disorders and autoimmune diseases.^{9,10} In the allergic condition mast cells are activated by cross-linking of receptor (Fc ϵ RI)-bound IgE by

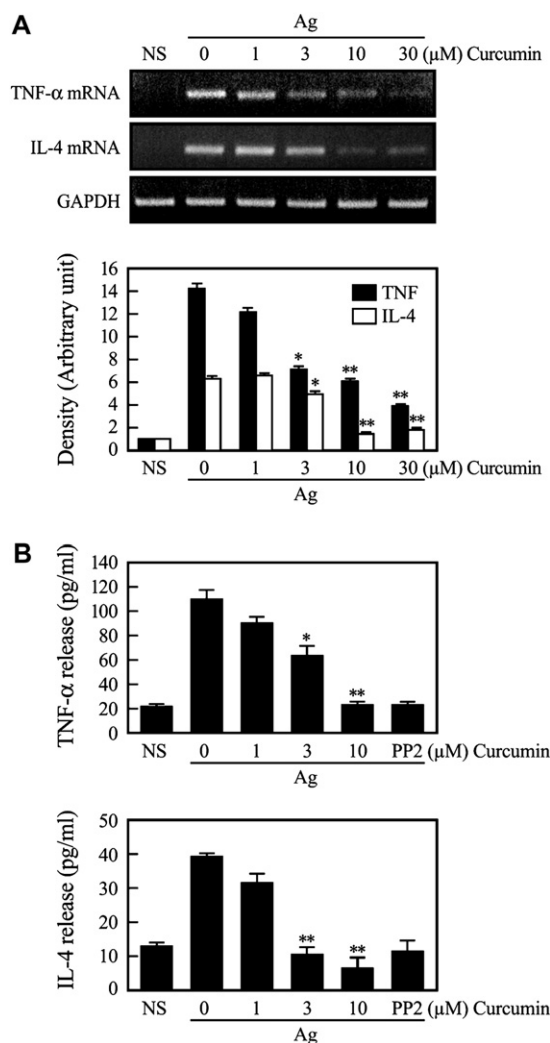


FIG 3. Curcumin suppresses the expression and secretion of TNF- α and IL-4. **A**, IgE-primed RBL-2H3 cells were stimulated with antigen or vehicle (NS) for RT-PCR. Densitometric values (means $\pm$ SEMs) for the 3 experiments are shown. **B**, TNF- α and IL-4 concentrations in the culture supernatant were determined by using commercial ELISA kits. Asterisks indicate significant differences from controls as follows: * P < .05 and ** P < .01. PP2 (10 μ mol/L) is a general Src-family kinase inhibitor. GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.

antigen. The ensuing aggregation of these receptors results in the rapid phosphorylation of tyrosine residues in the immunoreceptor tyrosine-based activation motifs of the Fc ϵ RI β and γ subunits by Lyn kinase,²⁴ which leads to recruitment and activation of the protein tyrosine kinase Syk.^{25,26} Syk is responsible for the phosphorylation and activation of a variety of downstream signaling molecules, among them LAT and Gab2, and in this manner regulates activation of phosphatidylinositol 3-kinase, phospholipase C, calcium mobilization, and MAP kinases.^{12,27} These signaling cascades regulate, in turn, degranulation and production of inflammatory lipids and cytokines, which elicit, coordinate, and sustain the allergic inflammatory response to antigen.¹¹ Therefore the development of mast cell inhibitors of Syk kinase might provide a reasonable approach toward therapeutic intervention in allergic disorders.

In this study we report that curcumin, a major constituent of turmeric and the coloring ingredient of curry, possesses

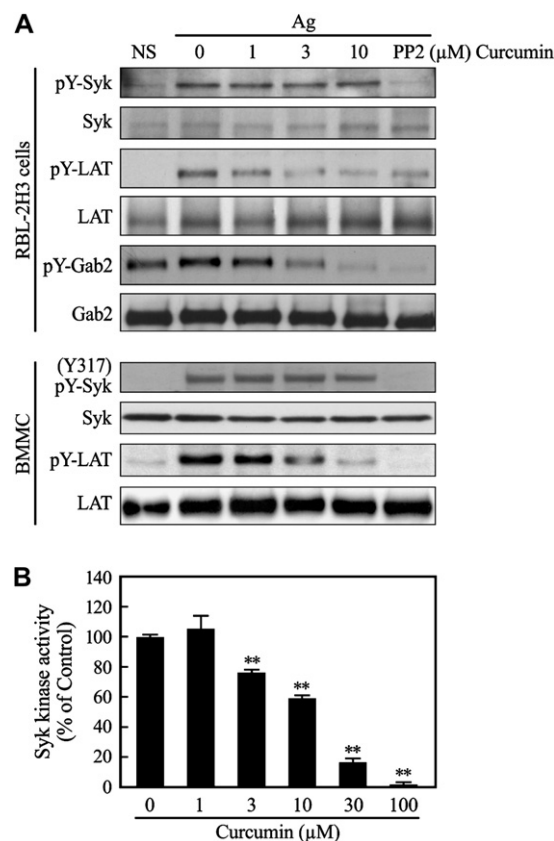


FIG 4. Inhibition of Syk-dependent phosphorylations and Syk kinase activity by curcumin. **A**, The mast cells were stimulated with DNP-BSA or not (NS) for 7 minutes. The proteins were immunoprecipitated from lysates of RBL-2H3 cells. BMMC lysates were used to detect phosphorylation of Syk and LAT specifically. **B**, *In vitro* Syk kinase assay with curcumin. Values indicate the means $\pm$ SEMs from 3 independent experiments: ** P < .01. PP2 (10 μ mol/L) is a general Src-family kinase inhibitor.

antiallergic activity in a mast cell-dependent model, most likely through its ability to potently inhibit Syk-mediated signals that lead to degranulation and cytokine secretion in antigen-stimulated mast cells. These effects are in addition to its previously reported immunosuppressive actions in animal models of latex allergy³ and allergen-induced airway hyperresponsiveness.² However, the cellular and molecular targets were not identified. Our finding that curcumin inhibited mast cell activation and mast cell-mediated anaphylaxis in mice prompted our examination of the effects of curcumin on key signaling events in 2 mast cell lines: RBL-2H3 and BMMCs. The key observation was that curcumin did not inhibit tyrosine phosphorylation of Syk in either cell line (Fig 4, A), but it did appear to suppress the activity of Syk itself.

Syk is initially phosphorylated on tyrosine 317 by Src-family kinases, primarily Lyn, the first kinase to be activated after antigen stimulation.^{22,23} Curcumin was found not to suppress phosphorylation of this particular tyrosine in Syk in BMMCs (Fig 4, A, lower panel). In contrast, PP2, a general Src-family kinase inhibitor, which was used as a control, potently inhibited phosphorylation of Syk Y317. However, the phosphorylation of the Syk downstream substrates LAT and Gab2 was significantly suppressed by curcumin in a dose-dependent manner (Fig 4, A). Furthermore, curcumin directly inhibited Syk kinase *in vitro*

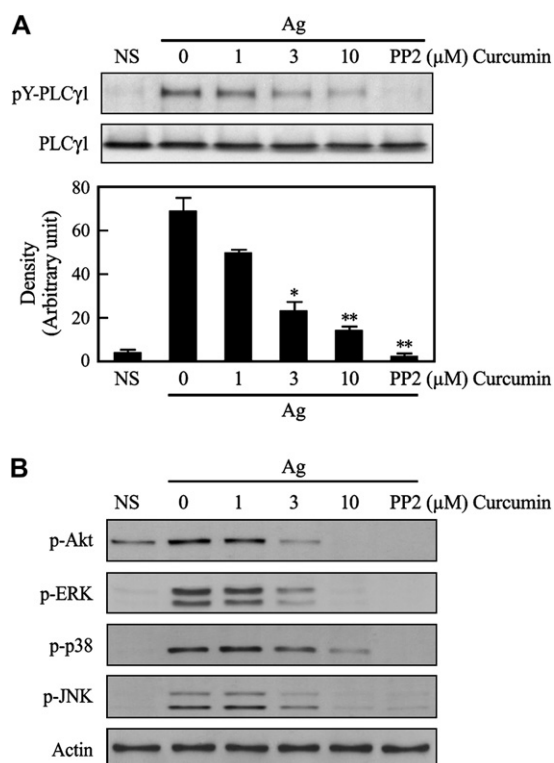


FIG 5. Curcumin suppresses phosphorylation of phospholipase C γ and MAP kinases. **A**, Immunoblots for phosphorylated and total phospholipase (PL) C γ 1 were prepared from immunoprecipitated PLC γ 1 in RBL-2H3 cells. Densitometric data (means $\pm$ SEMs) from 3 experiments are shown: * $P < .05$ and ** $P < .01$. **B**, Activating phosphorylations of Akt and the indicated MAP kinases were determined by preparation of immunoblots from whole cell lysates. PP2 (10 μ M) is a general Src-family kinase inhibitor.

(Fig 4, B). These results indicated that curcumin acts as an inhibitor of Syk kinase activation without interfering with its phosphorylation by Src-family kinases. As a consequence, it inhibits phosphorylation of signaling molecules downstream of Syk.^{28,29} The molecules affected included Akt/protein kinase B, a surrogate marker for phosphoinositide-3 kinase activation, extracellular signal-regulated kinase, p38, and c-Jun N-terminal kinase (Fig 5, B). Curcumin exhibited remarkable similarity in its potency in quenching activating phosphorylations of various effector molecules that have in common a requirement for upstream activation of Syk. Collectively, these results suggest that curcumin acts initially at the level of Syk kinase to suppress downstream signaling events and mast cell activation, although additional inhibitory actions on downstream signals cannot be excluded.

The PCA animal model used here is a well-established model for evaluating localized mast cell-mediated allergic reactions *in vivo*.²⁰ Curcumin effectively suppressed the local allergic reaction in this model in a dose-dependent manner (Fig 1). Substantial inhibition was observed with 0.5 mg/kg curcumin when administered orally. Near-complete suppression was observed with 50 mg/kg curcumin, and this suppression was comparable to that achieved with 50 mg/kg DPH. The question remains whether the tissue concentrations of curcumin after administration of 5 to 50 mg/kg (14–140 μ M/kg) curcumin are sufficient to suppress mast cell activation, namely 1 to 10 μ M/L curcumin *in vitro* (Figs 2, A, and 3).

Pharmacokinetic data in mice are limited but indicate that plasma concentrations of curcumin are sustained for several hours after oral administration and that curcumin accumulates in peripheral tissues after intraperitoneal administration of curcumin.³⁰

Curcumin is a pluripotent pharmacologic agent, which likely accounts for its broad biologic activities.¹ It downregulates transcription factors and inflammatory enzymes, such as nuclear factor κ B, activator protein 1, Egr-1, COX-2, 5-lipoxygenase, and inducible nitric oxide synthase. It also downregulates growth factor receptors, cell-surface adhesion molecules, and cyclin D1. In addition, it is reported to inhibit the activity of protein tyrosine kinases and protein kinase C in the 10- to 100- μ M/L range^{31,32} or concentrations higher than those required for the inhibition of Syk, as reported here. The molecular mechanisms for these actions are obscure but are thought to be due in part to the potent antioxidant properties of curcumin.³³ Curcumin is also a thiol-modifying reagent and can alkylate critical cysteine residues and thus irreversibly inhibit such enzymes as thioredoxin reductase³⁴ and IL-1 receptor-associated kinase,³⁵ a key kinase in Toll-like receptor signaling systems. However, cysteine modification is an unlikely mechanism for the suppression of Syk because such suppression is completely reversible (Fig 2, B). Despite its wide array of activities, curcumin has relatively low toxicity in human subjects.³⁶ Large doses of curcumin can cause gastrointestinal problems, including diarrhea and constipation and, in rare cases, contact dermatitis.^{37,38} In addition, curcumin disrupts the p53 tumor-suppressor pathway in colon cancer cells, and the potential exists for carcinogenic effects.³⁹

Syk inhibition has previously been shown to result in the reduction of allergic airway inflammation.^{17,40,41} Syk inhibitors are also being evaluated for use in nasal sprays for the treatment of symptoms of seasonal allergic rhinitis.^{42–44} In this respect curcumin has similar potential for topical therapy of various allergic diseases, including allergic rhinitis, especially in view of its low toxicity in human subjects. Curcumin is a potent inhibitor of antigen-stimulated degranulation and cytokine secretion in mast cells and of mast cell-mediated PCA in mice. A key action appears to be its ability to inhibit Syk kinase activation and downstream signals in mast cells. These and the known anti-inflammatory properties of curcumin suggest that further evaluation of its utility in the treatment of immediate and delayed allergic diseases is warranted.

We thank Dr Hyung Sik Kim (College of Pharmacy, Pusan University, Busan, Korea) for critical reading and comments.

Clinical implications: Dietary curry curcumin might ameliorate allergic diseases.

REFERENCES

1. Maheshwari RK, Singh AK, Gaddipati J, Simal RC. Multiple biological activities of curcumin: a short review. *Life Sci* 2006;78:2081–7.
2. Ram A, Das M, Ghosh B. Curcumin attenuates allergen-induced airway hyperresponsiveness in sensitized guinea pigs. *Biol Pharm Bull* 2003;26:1021–4.
3. Kurup VP, Barrios CS, Raju R, Johnson BD, Levy MB, Fink JN. Immune response modulation by curcumin in a latex allergy model. *Clin Mol Allergy* 2007;5:1–12.
4. Matsuda H, Tewtrakul S, Morikawa T, Nakamura A, Yoshikawa M. Anti-allergic principles from Thai zedoary: structural requirements of curcuminoids for inhibition of degranulation and effect on the release of TNF-alpha and IL-4 in RBL-2H3 cells. *Bioorg Med Chem* 2004;12:5891–8.

5. Suzuki M, Nakamura T, Iyoki S, Fujiwara A, Watanabe Y, Mohri K, et al. Elucidation of anti-allergic activities of curcumin-related compounds with a special reference to their anti-oxidative activities. *Biol Pharm Bull* 2005;28:1438-43.
6. Kay AB. Allergy and allergic diseases. First of two parts. *N Engl J Med* 2001;344:30-7.
7. Kay AB. Allergy and allergic diseases. Second of two parts. *N Engl J Med* 2001;344:109-13.
8. Luskova P, Draber P. Modulation of the Fcε receptor I signaling by tyrosine kinase inhibitors: search for therapeutic targets of inflammatory and allergy diseases. *Curr Pharm Des* 2004;10:1727-37.
9. Bischoff SC. Role of mast cells in allergic and non-allergic immune responses: comparison of human and murine data. *Nat Rev Immunol* 2007;7:93-104.
10. Gregory GD, Brown MA. Mast cells in allergy and autoimmunity: implications for adaptive immunity. *Methods Mol Biol* 2006;315:35-50.
11. Gilfillan AM, Tkaczyk C. Integrated signaling pathways for mast-cell activation. *Nat Rev Immunol* 2006;6:218-30.
12. Rivera J. Molecular Adapters in FcεRI signaling and the allergic response. *Curr Opin Immunol* 2002;14:688-93.
13. Rivera J, Gilfillan AM. Molecular regulation of mast cell activation. *J Allergy Clin Immunol* 2006;117:1214-25.
14. Andrade MV, Hiragun T, Beaven MA. Dexamethasone suppresses antigen-induced activation of phosphatidylinositol 3-kinase and downstream responses in mast cells. *J Immunol* 2004;172:7254-62.
15. Lorentz A, Klopp I, Gebhardt T, Manns MP, Bischoff SC. Role of activator protein 1, nuclear factor-κB, and nuclear factor of activated T cells in IgE receptor-mediated cytokine expression in mature human mast cells. *J Allergy Clin Immunol* 2003;111:1062-8.
16. Frossi B, Rivera J, Hirsch E, Pucillo C. Selective activation of Fyn/PI3K and p38 MAPK regulates IL-4 production in BMDC under nontoxic stress condition. *J Immunol* 2007;178:2549-55.
17. Wong BR, Grossbard EB, Payan DG, Masuda ES. Targeting Syk as a treatment for allergic and autoimmune disorders. *Expert Opin Investig Drugs* 2004;13:743-62.
18. Ozawa K, Szallasi Z, Kazanietz MG, Blumberg PM, Mischak H, Mushinski JF, et al. Ca²⁺-Dependent and Ca²⁺-independent isozymes of protein kinase C mediate exocytosis in antigen-stimulated rat basophilic RBL-2H3 cells: Reconstitution of secretory responses with Ca²⁺ and purified isozymes in washed permeabilized cells. *J Biol Chem* 1993;268:1749-56.
19. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage t4. *Nature* 1970;227:680-5.
20. Kabu K, Yamasaki S, Kamimura D, Ito Y, Hasegawa A, Sato E, et al. Zinc is required for FcεRI-mediated mast cell activation. *J Immunol* 2006;177:1296-305.
21. Theoharides TC, Kalogeromitros D. The critical role of mast cells in allergy and inflammation. *Ann N Y Acad Sci* 2006;1088:78-99.
22. Chu DH, Morita CT, Weiss A. The Syk family of protein tyrosine kinases in T-cell activation and development. *Immunol Rev* 1998;165:167-80.
23. Law CL, Chandran KA, Sidorenko SP, Clark EA. Phospholipase C-γ1 interacts with conserved phosphotyrosyl residues in the linker region of Syk and is a substrate for Syk. *Mol Cell Biol* 1996;16:1305-15.
24. Vonakis BM, Chen H, Haleem-Smith H, Metzger H. The unique domain as the site on Lyn kinase for its constitutive association with the high affinity receptor for IgE. *J Biol Chem* 1997;272:24072-80.
25. Zhang J, Berenstein EH, Evans RL, Siraganian RP. Transfection of Syk protein tyrosine kinase reconstitutes high affinity IgE receptor-mediated degranulation in a Syk-negative variant of rat basophilic leukemia RBL-2H3 cells. *J Exp Med* 1996;184:71-80.
26. El-Hillal O, Kurosaki T, Yamamura H, Kinet JP, Scharenberg AM. Syk kinase activation by a src kinase-initiated activation loop phosphorylation chain reaction. *Proc Natl Acad Sci U S A* 1997;94:1919-24.
27. Nadler MJ, Matthews SA, Turner H, Kinet JP. Signal transduction by the high-affinity immunoglobulin E receptor FcεRI: coupling form to function. *Adv Immunol* 2000;76:325-55.
28. Siraganian RP. Mast cell signal transduction from the high-affinity IgE receptor. *Curr Opin Immunol* 2003;15:639-46.
29. Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. *Nat Immunol* 2005;6:135-42.
30. Pan MH, Huang TM, Lin JK. Biotransformation of curcumin through reduction and glucuronidation in mice. *Drug Metab Dispos* 1999;27:486-94.
31. Liu JY, Lin SJ, Lin JK. Inhibitory effects of curcumin on protein kinase C activity induced by 12-O-tetradecanoyl-phorbol-13-acetate in NIH 3T3 cells. *Carcinogenesis* 1993;14:857-61.
32. Chen HW, Huang HC. Effect of curcumin on cell cycle progression and apoptosis in vascular smooth muscle cells. *Br J Pharmacol* 1998;124:1029-40.
33. Sandur SK, Ichikawa H, Pandey MK, Kunnumakkara AB, Sung B, Sethi G, et al. Role of pro-oxidants and antioxidants in the anti-inflammatory and apoptotic effects of curcumin (diferuloylmethane). *Free Radic Biol Med* 2007;43:568-80.
34. Fang J, Lu J, Holmgren A. Thioredoxin reductase is irreversibly modified by curcumin: a novel molecular mechanism for its anticancer activity. *J Biol Chem* 2005;280:25284-90.
35. Jürmann N, Brigelius-Flohe R, Bol GF. Curcumin blocks interleukin-1 (IL-1) signaling by inhibiting the recruitment of the IL-1 receptor-associated kinase IRAK in murine thymoma EL-4 cells. *J Nutr* 2005;135:1859-64.
36. Hsu CH, Cheng AL. Clinical studies with curcumin. *Adv Exp Med Biol* 2007;595:471-80.
37. Liddle M, Hull C, Liu C, Powell D. Contact urticaria from curcumin. *Dermatitis* 2006;17:196-7.
38. Thompson DA, Tan BB. Tetrahydracurcumin-related allergic contact dermatitis. *Contact Dermatitis* 2006;55:254-5.
39. Moos PJ, Edes K, Mullally JE, Fitzpatrick FA. Curcumin impairs tumor suppressor p53 function in colon cancer cells. *Carcinogenesis* 2004;25:1611-7.
40. Matsubara S, Li G, Takeda K, Loader JE, Pine P, Masuda ES, et al. Inhibition of spleen tyrosine kinase prevents mast cell activation and airway hyperresponsiveness. *Am J Respir Crit Care Med* 2006;173:56-63.
41. Yamamoto N, Takeshita K, Shichijo M, Kokubo T, Sato M, Nakashima K, et al. The orally available spleen tyrosine kinase inhibitor 2-[7-(3,4-dimethoxyphenyl)-imidazo[1,2-c] pyrimidin-5-ylamino]nicotinamide dihydrochloride (BAY 61-3606) blocks antigen-induced airway inflammation in rodents. *J Pharmacol Exp Ther* 2003;306:1174-81.
42. Guyer BJ, Shimamoto SR, Bradhurst AL, Grossbard EB, Dreskin SC, Nelson HS. Mast cell inhibitor R112 is well tolerated and affects prostaglandin D2 but not other mediators, symptoms, or nasal volumes in a nasal challenge model of allergic rhinitis. *Allergy Asthma Proc* 2006;27:208-13.
43. Meltzer EO, Berkowitz RB, Grossbard EB. An intranasal Syk-kinase inhibitor (R112) improves the symptoms of seasonal allergic rhinitis in a park environment. *J Allergy Clin Immunol* 2005;115:791-6.
44. Masuda ES, Schmitz J. Syk inhibitors as treatment for allergic rhinitis. *Pulm Pharmacol Ther* 2007 [Epub ahead of print].