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Curcumin Inhibition of *Dermatophagoides farinea*-Induced Interleukin-5 (IL-5) and Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) Production by Lymphocytes from Bronchial Asthmatics

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ABSTRACT. Curcumin, a dietary pigment responsible for the yellow color of curry, has been used for the treatment of inflammatory diseases and exhibits a variety of pharmacological effects such as anti-inflammatory, anti-tumor, anti-oxidant, and anti-viral activity. However, it has not been determined whether the effect of curcumin on the production of cytokine affects eosinophil functions and IgE synthesis. In the present study, we examined the effect of curcumin on the production of interleukin (IL)-2, IL-5, granulocyte macrophage-colony stimulating factor (GM-CSF), and IL-4 by lymphocytes from atopic asthmatics in response to house dust mites (*Dermatophagoides farinea*: *Df*) in order to clarify a potential application for allergic diseases. Curcumin inhibited *Df*-induced lymphocyte proliferation and production of IL-2. Exogenous IL-2 reconstituted the proliferative responsiveness of lymphocytes to *Df* in the presence of curcumin. Furthermore, curcumin inhibited IL-5, GM-CSF, and IL-4 production in a concentration-dependent manner. These results indicate that curcumin may have a potential effect on controlling allergic diseases through inhibiting the production of cytokines affecting eosinophil function and IgE synthesis. BIOCHEM PHARMACOL **54**;7:819–824, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. curcumin; bronchial asthma; IL-2; IL-5; GM-CSF; IL-4

Curcumin, a dietary pigment responsible for the yellow color of curry, has been shown to exhibit anti-inflammatory [1], anti-tumor [2, 3], anti-oxidant [4, 5], and anti-viral [6] activity. In the modulatory effects of curcumin on immune functions, inhibitory effects of curcumin on lymphocyte proliferation [7], monocyte chemotactic protein production by osteoblastic cells [8], and tumor necrosis factor-α production by the human monocytic macrophage cell line Mono Mac 6 [9] have been shown. Consequently, curcumin may have a potential efficacy in controlling inflammatory disease. Indeed, curcumin has been used for the treatment of inflammatory diseases in certain countries [10].

Bronchial asthma is a disease that is characterized by episodic reversible airway obstruction, airway hyperresponsiveness, and allergic inflammation in the airway [11]. In the production of allergic inflammation, T lymphocytes are thought to orchestrate eosinophilic inflammation in bronchial asthma through the release of cytokines [12–14]. Of the cytokines produced by activated T cells, IL-5†, GM-CSF, and IL-3 are particularly implicated in the production of allergic inflammation, since these cytokines exhibit a potentially important influence on eosinophilic inflammation by promoting differentiation, growth, and survival of eosinophils, by enhancing adhesion of eosinophils to vascular endothelial cells, and by activating eosinophils [15–20]. Consequently, a suppression of cytokine production affecting eosinophil functions is an important strategy for the treatment of bronchial asthma [21, 22].

Although curcumin has been used for the treatment of inflammatory diseases and its mode of action has been examined, little is known about the effect of curcumin on cytokine production by lymphocytes. In the present work, we studied the effect of curcumin on the production of cytokines, which affect eosinophil functions by lymphocytes from patients with atopic bronchial asthma, in response to *Df* in order to examine a potential application of curcumin in the treatment of allergic diseases. In addition, we also examined the effect of curcumin on IL-4 production since a crucial role of IL-4 in IgE synthesis has been well documented [23].

MATERIALS AND METHODS

Subjects

The study group consisted of 8 patients with atopic bronchial asthma with a mean age of 32.2 years (range 28 to 38),

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† *Abbreviations: Df, Dermatophagoides farinea*; GM-CSF, granulocyte macrophage-colony stimulating factor; and IL, interleukin.

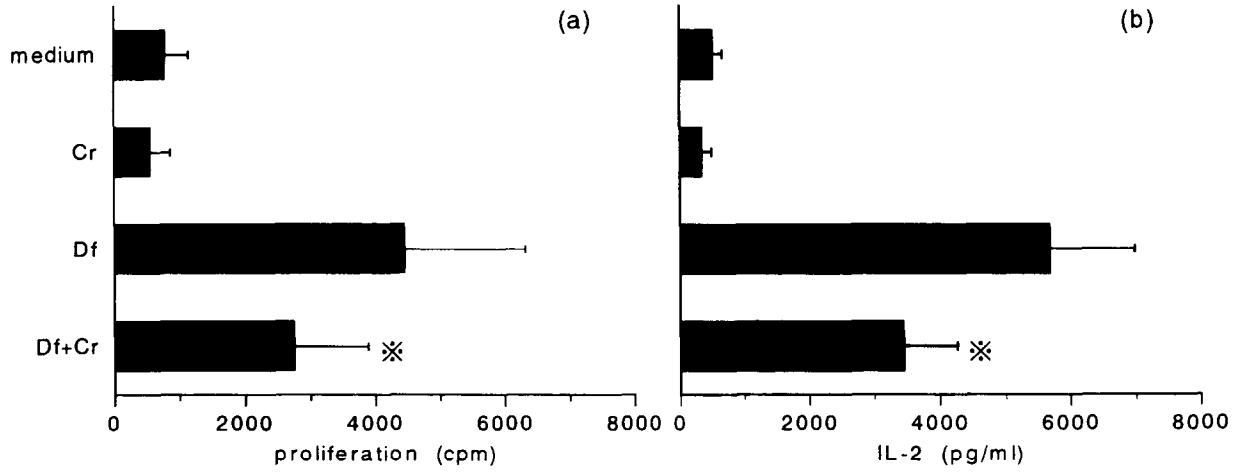

FIG. 1. Effect of curcumin on the proliferative response and IL-2 production by lymphocytes stimulated with *Df*. Lymphocytes from atopic asthmatics were stimulated with *Df* in the presence or absence of curcumin (Cr, 10 μM). Lymphocyte proliferation (Fig. 1a) and the concentrations of IL-2 (Fig. 1b) in *Df*-stimulated cultures were determined on day 7 and on day 3 after cultivation, respectively. The results are expressed as the means ± SD in eight different subjects. Key: (*)$P < 0.05$ compared with *Df*-induced proliferation or IL-2 production in the absence of curcumin.

5 patients with non-atopic bronchial asthma with a mean age of 34.6 years (range 28 to 42), and 8 healthy normal subjects with a mean age of 32.4 years (range 25 to 43 years). All patients with bronchial asthma met the American Thoracic Society's definition of asthma [11]. We emphasize that all asthmatic patients had a history of episodic wheezing and dyspnea, reversible bronchoconstriction, and airway hyperresponsiveness measured by directly writing the dose–response curve of respiratory resistance during the continuous inhalation of methacholine in stepwise incremental concentrations [24]. Atopic or non-atopic bronchial asthma was defined by a history of bronchoconstrictive response after allergen exposure, total serum IgE levels (>250 IU/mL), and specific IgE levels against *Df* [>0.34 Phadebas RAST Unit (PRU)/mL] and/or skin prick tests to *Df*. At the time of this study, none of the patients were taking inhaled and oral corticosteroids. The severity of asthma was defined as follows: mild, dyspnea attacks less than three times a week; moderate, dyspnea attacks more than three times a week; and severe, daily dyspnea attacks. All patients were mild cases. Blood samples from patients with bronchial asthma were taken in stable conditions. Asthma attacks and stable conditions (asymptomatic period) were defined on the basis of the presence of clinical symptoms such as wheezing and chest tightness, and values of peak expiratory flow rate (PEFR). The patients had wheezing and chest tightness, and decreased values of PEFR during asthma attacks, whereas they were asymptomatic and their PEFR values were greater than 70% of the predicted in stable conditions. Informed consent was obtained from all patients and normal control subjects.

Materials

Curcumin was obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). *Df* and human recombinant IL-2 was provided by the Torii Pharmaceutical Co. Ltd. (Tokyo, Japan) and the Shionogi Pharmaceutical Co. Ltd. (Osaka, Japan), respectively.

Cell Preparation

Human peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by centrifugation over Ficoll–Hypaque gradients. The PBMC were washed three times and resuspended in HEPES-buffered RPMI 1640 (Irvine, Santa Ana, CA, U.S.A.) supplemented with 10% heat-inactivated fetal bovine serum (Mitsubishikasei, Tokyo, Japan), streptomycin, and penicillin (Meiji Pharmaceutical, Tokyo, Japan).

Cell Culture

PROLIFERATION ASSAY. PBMC (2×10^5 cells/well in 0.2 mL) were cultured in flat-bottomed microplates (Costar, Cambridge, MA, U.S.A.) with *Df* (10 μg/mL) in the presence or absence of curcumin at 37° in a humidified 5% CO_2 atmosphere. Tritiated thymidine was added to cell culture wells for the last 4 hr of the 7-day incubation period for *Df*-stimulated culture. After incubation, the cells were harvested onto glass fiber strips with a cell harvester, and retained radioactivity was counted in a scintillation counter. In the experiment designed to determine the effect of exogenous IL-2 on lymphocyte proliferation, IL-2 (100 ng/mL) was added at the initiation of culture.

CYTOKINE PRODUCTION. PBMC were stimulated with *Df* in the presence or absence of curcumin and cultured in tubes (Falcon 2003; 1×10^6 cells/tube) containing 1 mL of culture medium at 37° in a humidified 5% CO_2 atmosphere for

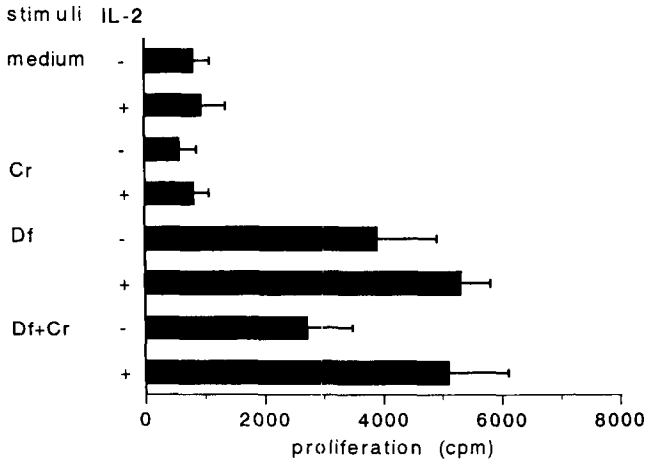

FIG. 2. Reconstitution by exogenous IL-2 of the proliferative response inhibition induced by curcumin. Lymphocytes from atopic asthmatics stimulated with *Df* in the presence or absence of curcumin (Cr, 10 μM) were cultured with or without exogenous IL-2 (100 ng/mL), and lymphocyte proliferation was determined on day 7 after cultivation. The results are expressed as the means ± SD in five different subjects.

various time periods as specified in the Results. At the end of culture, supernatants were harvested and stored at −80°.

Measurement for Concentrations of Cytokine in the Culture Supernatants

Concentrations of IL-2, IL-5, GM-CSF, and IL-4 were measured by commercially available ELISA kits (T Cell Science Inc., Cambridge, MA, U.S.A.). ELISA was performed according to the manufacturer's instructions.

Statistical Analysis

Statistical significance was analyzed using Student's *t*-test. *P* values less than 0.05 were considered significant.

RESULTS

Effect of Curcumin on the Proliferative Response and IL-2 Production of Lymphocytes Stimulated with Df

In the initial experiments, we examined the effect of curcumin on the *Df*-induced proliferative response of lymphocytes from patients with bronchial asthma. In addition, since IL-2 is a major cytokine controlling the proliferation of lymphocytes, we also examined the effect of curcumin on the production of IL-2. To this end, lymphocytes from patients with atopic bronchial asthma were stimulated with *Df* in the presence or absence of curcumin.

Lymphocyte proliferation on day 7 after cultivation and IL-2 concentrations in the culture supernatants on day 3 were measured. Curcumin inhibited *Df*-induced lymphocyte proliferation (Fig. 1a) and IL-2 production (Fig. 1b). When lymphocytes from patients with non-atopic bronchial asthma and normal healthy subjects were stimulated with *Df*, no lymphocyte proliferation and IL-2 production were observed (data not shown).

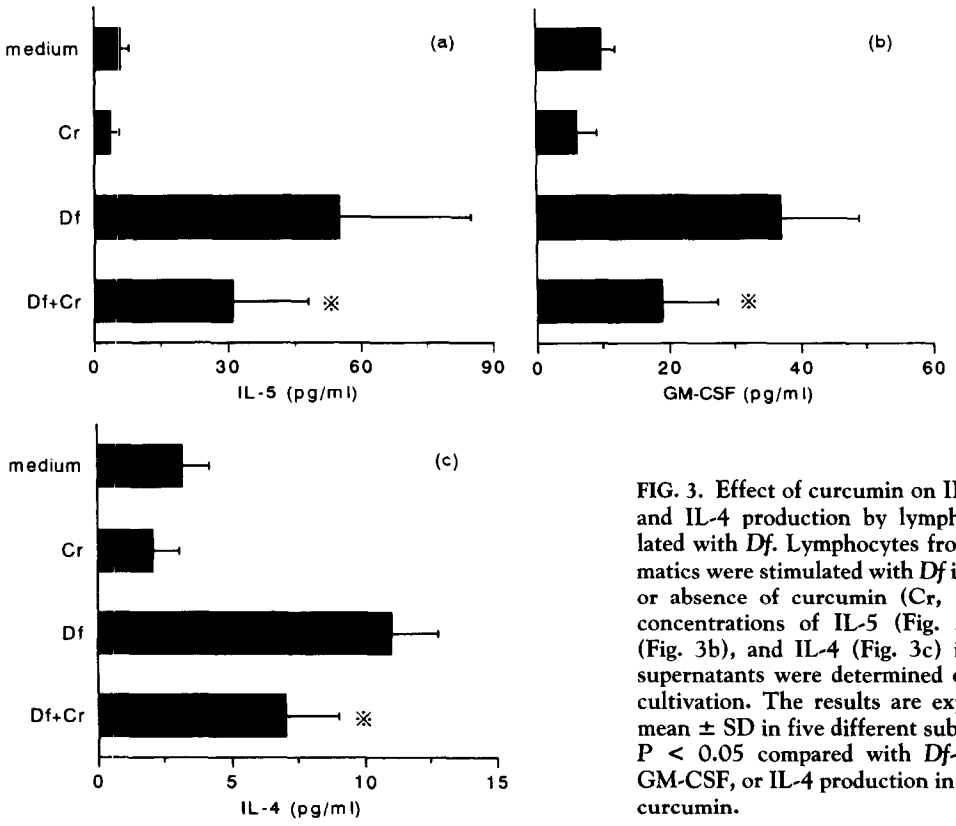

FIG. 3. Effect of curcumin on IL-5, GM-CSF, and IL-4 production by lymphocytes stimulated with *Df*. Lymphocytes from atopic asthmatics were stimulated with *Df* in the presence or absence of curcumin (Cr, 10 μM). The concentrations of IL-5 (Fig. 3a), GM-CSF (Fig. 3b), and IL-4 (Fig. 3c) in the culture supernatants were determined on day 3 after cultivation. The results are expressed as the mean ± SD in five different subjects. Key: (*) $P < 0.05$ compared with *Df*-induced IL-5, GM-CSF, or IL-4 production in the absence of curcumin.

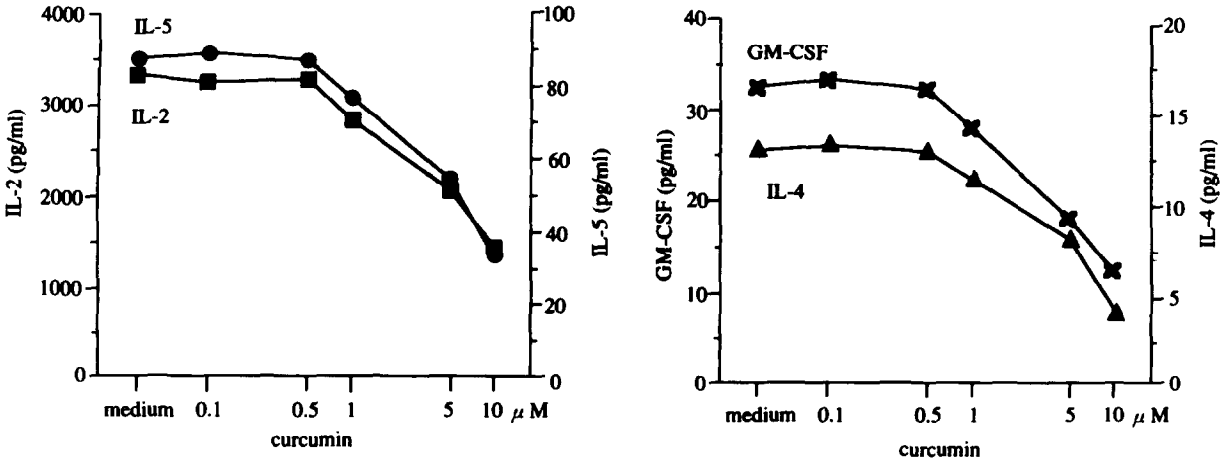

FIG. 4. Concentration-related inhibition by curcumin of cytokine production by lymphocytes stimulated by *Df*. Lymphocytes from atopic asthmatics were stimulated with *Df* in the presence or absence of several concentrations of curcumin. The concentrations of IL-2 (■), IL-5 (●), GM-CSF (X), and IL-4 (▲) in the culture supernatants on day 3 were determined. The results are representative of two different subjects.

Reconstitution by Exogenous IL-2 of the Proliferative Response Inhibition Induced by Curcumin

If the reduction in IL-2 measured on day 3 after cultivation in curcumin-treated cultures is primarily responsible for the inhibited proliferation on day 7, then the proliferative response would be expected to be reconstituted by the addition of exogenous IL-2. Indeed, we found exogenous IL-2 reconstituted the proliferative responsiveness of lymphocytes to *Df* in the presence of curcumin (Fig. 2).

Effect of Curcumin on IL-5, GM-CSF, and IL-4 Production by Lymphocytes Stimulated with Df

Lymphocytes from patients with atopic bronchial asthma were stimulated with *Df* in the presence or the absence of curcumin and cultured for 3 days, and the concentrations of IL-5, GM-CSF, and IL-4 were measured to determine the effect of curcumin on the production of these cytokines. The accumulation of IL-5 over 3 days in *Df*-stimulated cultures was inhibited markedly by curcumin. Similarly, the concentrations of GM-CSF and IL-4 in *Df*-stimulated cultures in the presence of curcumin were lower than those in the absence of curcumin, indicating that curcumin inhibited the production of IL-5 (Fig. 3a), GM-CSF (Fig. 3b), and IL-4 (Fig. 3c), as well as IL-2.

Concentration-related Inhibition by Curcumin of Cytokine Production by Lymphocytes Stimulated by Df

Addition of curcumin to cultured lymphocytes at a concentration of 0.1 to 10 μM resulted in a concentration-dependent inhibition of *Df*-induced production of IL-2, IL-5, GM-CSF, and IL-4 (Fig. 4).

Kinetics of Inhibition of IL-2, IL-5, GM-CSF, and IL-4 Accumulation by Curcumin

The concentrations of IL-2, IL-5, GM-CSF, and IL-4 in the culture supernatants from lymphocytes stimulated with *Df* in the presence or the absence of curcumin were measured at 1-day intervals from day 1 to day 4. As shown in Fig. 5, the concentrations of IL-2 from lymphocytes stimulated with *Df* in the presence of curcumin were lower than those in the absence of curcumin at any time of culture. Similar observations were obtained in IL-5, GM-CSF, and IL-4. At the end of the culture period, cell viability as determined by the trypan blue exclusion assay did not differ with the culture conditions indicated in Figs. 1 through 5.

DISCUSSION

Our results showed that curcumin inhibited the proliferative response of lymphocytes and the production of IL-2, IL-5, GM-CSF, and IL-4 by *Df*-stimulated lymphocytes.

Curcumin inhibited *Df*-induced proliferation and production of IL-2 by lymphocytes. The correlation between the inhibition of proliferation and IL-2 production by curcumin, as well as the reconstitution of the inhibited proliferative response by exogenous IL-2, suggests that, at least in this system, the primary role played by curcumin is in the events that lead to the production of (rather than responsiveness to) this lymphokine. The reconstitution of the inhibited proliferative response by exogenous IL-2 and the trypan blue exclusion assay indicated that the 10 μM concentration of curcumin used in this study did not have a toxic effect on lymphocyte functions.

There are several mechanisms by which curcumin inhibits lymphocyte proliferation and lymphokine production. The promoter of the gene encoding IL-2, IL-5, GM-CSF, and IL-4 contains sequences for binding of several nuclear transcription factors including AP-1, NF-κB, and NF-AT [25–29]. These transcription factors participate to various extents in the inducible expression of the genes encoding cytokines. Although curcumin has been shown to inhibit the activation of c-Jun/AP-1 in osteoblastic cells [8] and mouse fibroblasts [30], and of NF-κB in a human monocytic

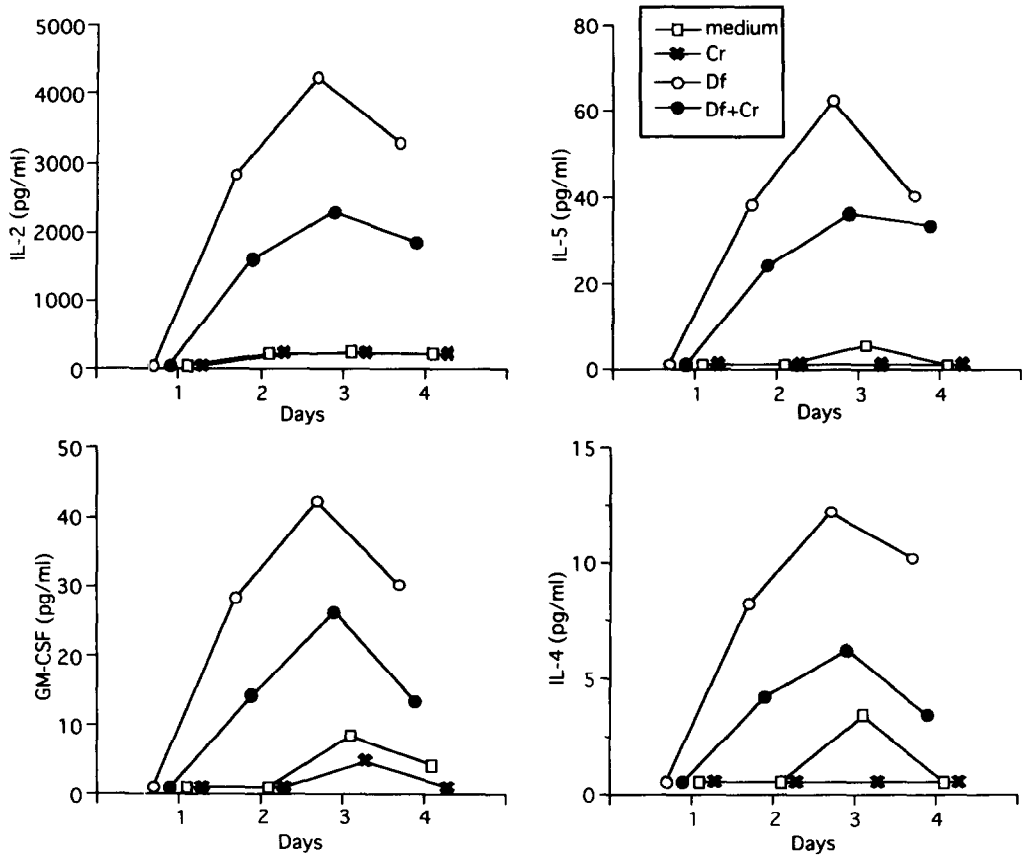

FIG. 5. Kinetics of inhibition of IL-2, IL-4, IL-5, and GM-CSF accumulation by curcumin. Lymphocytes from atopic asthmatics were cultured either with medium (□), curcumin (Cr, 10 μM, X), *Df* (○), or *Df* and Cr (●). The concentrations of IL-2, IL-5, GM-CSF, and IL-4 in the culture supernatants on days 1, 2, 3, and 4 were determined. The results are representative of two different subjects.

macrophage cell line [9, 31], there is no direct evidence in the literature for such a mechanism in lymphocytes. Another possibility is the inhibition of intracellular signals including protein kinase C by curcumin, since curcumin has been shown to inhibit protein kinase C activation [32]. In the present study, we shed light on the effect of curcumin on cytokine production by lymphocytes and did not examine the mechanism in curcumin-mediated inhibition of cytokine production. The precise mechanism in curcumin-mediated inhibition of cytokine production by lymphocytes remains to be clarified.

Inhaled and oral corticosteroids have been well documented to be effective in reducing airway inflammation and controlling asthmatic symptoms [33, 34], but some patients with chronic severe asthma are dependent on or refractory to their effects despite oral therapy [35, 36]. In the past few years, the effects of several anti-inflammatory agents and immunomodulators, including cyclosporin and methotrexate, on controlling asthmatic symptoms and reducing the dependence on high-dose corticosteroid therapy have been investigated [37–39]. The effects of these agents are often thought to be associated with a reduction in mRNA expression and production of cytokines affecting eosinophil functions [34, 36, 39, 40]. We currently are examining the effect of curcumin on cytokine mRNA levels, airway eosinophilia, and airway hyperresponsiveness in mice.

From data presented here, we conclude that curcumin exhibits an inhibitory effect on IL-5, GM-CSF, and IL-4 production. These results indicate that curcumin may have a potential effect on controlling allergic diseases through inhibiting the production of cytokines affecting eosinophil function and IgE synthesis. However, the precise effect of curcumin on allergic diseases remains to be clarified.

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