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Effect of thymoquinone on cyclooxygenase expression and prostaglandin production in a mouse model of allergic airway inflammation

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Abstract

Prostaglandins (PGs) are potent proinflammatory mediators generated through arachidonic acid metabolism by cyclooxygenase-1 and -2 (COX-1 and COX-2) in response to different stimuli and play an important role in modulating the inflammatory responses in a number of conditions, including allergic airway inflammation. Thymoquinone (TQ) is the main active constituent of the volatile oil extract of *Nigella sativa* seeds and has been reported to have anti-inflammatory properties. We examined the effect of TQ on the in vivo production of PGs and lung inflammation in a mouse model of allergic airway inflammation. Mice sensitized and challenged through the airways with ovalbumin (OVA) exhibited a significant increase in PGD2 and PGE2 production in the airways. The inflammatory response was characterized by an increase in the inflammatory cell numbers and Th2 cytokine levels in the bronchoalveolar lavage (BAL) fluid, lung airway eosinophilia and goblet cell hyperplasia, as well as the induction of COX-2 protein expression in the lung. Intraperitoneal injection of TQ for 5 days before the first OVA challenge attenuated airway inflammation as demonstrated by the significant decrease in Th2 cytokines, lung eosinophilia, and goblet cell hyperplasia. This attenuation of airway inflammation was concomitant to the inhibition of COX-2 protein expression and PGD2 production. However, TQ had a slight inhibitory effect on COX-1 expression and PGE2 production. These findings suggest that TQ has an anti-inflammatory effect during the allergic response in the lung through the inhibition of PGD2 synthesis and Th2-driven immune response.
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Keywords: Inflammation; Allergy; Prostaglandins; Cyclooxygenases; Th2 cytokines; Thymoquinone

1. Introduction

Persistent mucosal airway inflammation, associated with an increase in T helper type 2 (Th2) cytokine levels, eosinophil infiltration into the airways, mucus and immunoglobulin (Ig) E production, is the main feature of allergic asthma [1–8]. Prostaglandins (PGs) are potent proinflammatory mediators derived from arachidonic acid metabolism by cyclooxygenases (COXs), and play an important role in modulating a number of pathophysiological conditions, including inflammatory and allergic immune response [9]. The two isoforms of COX enzymes have been well studied. COX-1 is constitutively expressed and plays an important role in maintaining the normal physiological function of cells, and COX-2 whose expression is markedly induced by a number of stimuli, including cytokines, during the inflammatory response (reviewed in [10–12]). Information regarding the role of COXs and their derived PGs in allergic airway inflammation has been controversial. While some studies reported that PGs play a role in the pathogenesis of airway inflammation and asthma [13–15], others demonstrated that blocking PG production through the inhibition of COXs enhances the inflammatory response [16–18].

Prostaglandin D2 (PGD2) is the major product of arachidonic acid cyclooxygenation and is produced by inflammatory cells in the airways [14,15,19], as well as activated Th2 cells and mast cells [20,21]. Overproduction of PGD2 results in an increased eosinophilic airway inflammation and Th2 cytokine production in allergic mice [22]. PGD2 mediates its effects by binding to

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prostanoid receptors (DP) 1 and 2 expressed on Th2 cells and other inflammatory cells [23–25]. In addition, activation of DP2 results in increased airway inflammation [26], and mice deficient in DPs have reduced airway inflammation [27].

Prostaglandin E2 (PGE2) is produced at high levels in the lung by epithelial and smooth muscle cells, and play a protective role during the allergic immune response by inhibiting mast cells degranulation and eosinophils chemotaxis and survival (reviewed in [10,11]). In addition, inhalation of PGE2 reduces the inflammatory cell infiltration into the airways of asthmatic subjects [28]. Some studies using murine models of allergic airway inflammation suggest that PGE2 augments Th2 inflammation by inhibiting Th1 cytokines such as IFN-γ [29], while others suggest that PGE2 attenuates Th2-mediated inflammation by inhibiting Th2 cytokine and IgE production [30,31]. This information suggests that PGE2 plays an immunomodulatory role during the allergic response in the lung.

Previous studies have shown that *Nigella sativa* seeds and its oil extract possess anti-tumor [32], anti-oxidant [33], and anti-inflammatory activities in a number of diseases, including bronchial asthma [34–39]. For example, Mahgoub [38] has shown that TQ administered orally at 10 mg/kg protected against experimentally-induced colitis in rats. El-Mahmoudy et al. [39] reported that TQ administered intraperitoneally in diabetic rats at 3 mg/kg or added to cultured macrophages at 10 μM normalized the elevated levels of the proinflammatory cytokines IL-1β and TNF-α. Kalus et al. [40] reported a decrease in the disease scores (i.e. IgE and eosinophil levels) in patients with allergic rhinitis, bronchial asthma and eczema after oral administration of *Nigella sativa* oil at 40–80 mg/kg/day for 8 weeks. Al-Majed et al. [41] have shown that TQ induced relaxation of guinea pigs isolated trachea by abolishing the effect of histamine and serotonin, which support a protective role for the seeds and its derived thymoquinone in broncial asthma. It has also been reported that the seeds and its oil extract are minimally toxic [34]. In addition, the volatile oil and its main active principle, TQ, have been shown to inhibit arachidonic acid metabolism and in vitro eicosanoid generation by rat peritonial leukocytes, through the inhibition of cyclooxygenase activity [35]. This information suggests that TQ may have inhibitory effects on the allergic inflammatory response in the lung through the inhibition of PG biosynthesis. However, there are no data concerning the in vivo action of TQ in a model of allergic pulmonary inflammation.

The present study was designed to investigate the potential anti-inflammatory role of TQ by examining its effect on cyclooxygenase expression and prostaglandin production in vivo in a mouse model of allergic airway inflammation.

2. Materials and methods

2.1. Mice

Males and females BALB/c mice 8–10 weeks of age were kept on 12-h light/12-h dark cycle with free access to an OVA-free diet.

2.2. Experimental protocol

Induction of allergic airway inflammation was performed by intraperitoneal (i.p.) sensitization and airway challenge through nasal inhalation. Mice (3 males and 3 females/group) were sensitized on days 1 and 14 by i.p. injection of 20 μg of OVA (Grade V; Sigma–Aldrich, St. Louis, MO) emulsified in 2 mg $Al(OH)_3$ (adjuvant) (Pierce Biotechnology, Rockford, IL) in a total volume of 0.1 ml saline. Two weeks after the second sensitization, mice were airway challenged three times (days 28–30) via nasal inhalation with 1% OVA/saline aerosol for 20 min, using a De Vilbiss nebulizer (Sunrise Medical, Carlsbad, CA). This protocol was previously shown to induce airway eosinophilic inflammation, Th2 cytokines and mucus production, and airway hyperreactivity [42,43]. For TQ (2-isopropyl-5-methyl-1,4-benzoquinone) treatment, sensitized mice were injected (i.p.) once daily for the 5 days preceding the first OVA challenge, with 3 mg/kg TQ (Sigma–Aldrich) in 10% DMSO or an equal volume of DMSO alone. All mice were analyzed 24 h after the last OVA challenge. The mice groups are described as follows: Naïve, mice sensitized and challenged with saline and injected with DMSO alone; S/Ch, mice sensitized and challenged with OVA and injected with DMSO alone; and S/Ch-TQ, mice sensitized and challenged with OVA but received five injections of TQ in DMSO before the first OVA challenge. Each experiment was repeated at least three times.

2.3. Bronchoalveolar lavage (BAL)

Mice were anesthetized and lungs were lavaged by instillation and withdrawal of 1 ml PBS through a tracheal cannula, and BAL fluid (BALF) was collected. Total BAL cells were determined with crystal violet staining using a hemocytometer. For differential cell count, cytocentrifuged preparations were fixed and stained with Diff-Quick (Dade Behring, Dudingen, Switzerland) and differentiated morphologically by counting 300 cells/slide.

2.4. Measurement of prostaglandins and Th2 cytokines in BAL fluid

PGD2 level was measured using EIA kit according to the manufacturer's protocol (Cayman, Ann Arbor, MI). PGE2 was measured with an ELISA kit (R&D Systems, Minneapolis, MN). The detection limit was 6 pg/ml for PGD2 and 13.4 pg/ml for PGE2. Levels of IL-4, IL-5, and IL-13 in BAL fluid were measured using ELISA kits (R&D Systems) according to the manufacturer's instructions, and were compared with known standards. Briefly, microtiter plates were coated overnight at 4 °C with the capture antibody, washed and then blocked at room temperature for 1 h. Samples and a series of diluted standards were incubated overnight at 4 °C. After washing, biotin-conjugated detection antibodies and avidin–HRP were added and left at room temperature for 1 h, followed by adding the substrate solution. The reactions were stopped after 30–40 min, and then read at 450 nm. The lowest detection limits were 7.8 pg/ml for IL-4 and IL-13, and 15.5 pg/ml for IL-5.

2.5. Isolation of lung cells

Lung cells were prepared after cannulating the trachea and perfusing the airways with cold PBS to collect BAL fluid. Lungs were then sliced and incubated in 5 ml PBS containing 1.6 mg/ml collagenase (CLS4; Invitrogen, Carlsbad, CA), antibiotics and 10% FBS at 37 °C for 1 h. The digested tissue was disrupted into a single cell suspension by passing through 20-gauge needle and filtered through a 45 μm nylon mesh. The resulting cell suspension was centrifuged at 1500 rpm for 5 min at 4 °C. Cells (whole lung cells) were washed in PBS, and used for RT-PCR and Western blot.

2.6. Western blot analysis

Whole lung cells (1.5×10^6) were washed with PBS and lysed by brief sonication in a buffer containing 50 mM HEPES (pH 7.9), 150 mM NaCl, 1% Triton X-100, 1 mM $MgCl_2$, 1 mM EDTA, 1 mM PMSF, 10% glycerol, 10 μg/ml aprotonin, and 1 mM leupeptin. Lysates were centrifuged for 5 min at 10,000 rpm and 4 °C, and the total protein concentration in the supernatants was determined by Bradford assay (Bio-Rad, Hercules, CA). Proteins (75 μg/lane) were resolved on 12% Tris–glycine denaturing SDS-PAGE, and transferred onto a nitrocellulose membranes (Amersham, Piscataway, NJ) at 100 V for 1 h in a buffer containing 25 mM Tris–HCl (pH 8.0), 192 mM glycine, and 20% methanol. Membranes were blocked in 20 ml of TBS (Tris-buffered saline) plus 0.1% Tween 20 (TBST) and 5% nonfat dry milk for 1 h at room temperature. After three washes in TBST (5 min each), membranes were incubated with rabbit anti-mouse COX-1 or COX-2 antibody (Chemicon, Temecula, CA) overnight at 4 °C. Blots were washed three times in TBST and incubated for 1 h at room temperature with the horseradish peroxidase-conjugated anti-mouse secondary antibody (Amersham). The blots were then washed three times in TBST and incubated for 1–2 min with 5 ml of enhanced chemiluminescence detection reagent (Amersham) at room temperature. The bands were visualized by exposure to Kodak X-AR film (Eastman Kodak, NY). Membranes were subsequently stripped in a buffer containing 62 mM Tris–HCl (pH 6.8), 20% SDS, and 100 mM β-mercaptoethanol and re-probed for GAPDH.

2.7. RT-PCR

Total RNA was extracted from whole lung cells using TRIzol reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA). RNA (1 μg) was then reverse-transcribed into cDNA using random hexamer primer and the Superscript First Strand Synthesis System (Invitrogen, Carlsbad, CA). The reaction was performed for 45 min at 37 °C in a total volume of 50 μl. For cDNA amplification, PCR was performed in 50 μl volumes with 0.5 μM total concentration of the cytokine primer pair. DNA was amplified for 35 cycles at 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, using 1.5 U of Taq DNA polymerase (Roche Diagnostics, Indianapolis, IN). Equal amounts of PCR products were run on 1.5% agarose gel and detected by ethidium bromide staining. GAPDH gene was amplified in the same manner and used as an internal control to normalize the synthesized products. Primer sequences were as follows: IL-4 sense, 5′-TCGGCATTTTGAACGAGGTC-3′ and antisense, 5′-GAAAAGCCCGAAAGAGTCTC-3′; IL-5, sense 5′-TCACCGAGCTCTGTTGACAA-3′ and antisense, 5′-CCACACTTCTCTTTTTGGCG-3′; IL-13 sense, 5′-GACCCAGAGGATATTGCATG-3′ and antisense, 5′-CCAGCAAAGTCTGATGTGAG-3′; GAPDH sense, 5′-TTCACCACCATGGAGAAGGC-3 and antisense, 5′-GGCATGGACTGTGGTCATGA.

2.8. Determination of OVA-specific antibodies

Blood was collected by cardiac puncture, and serum levels of OVA-specific IgE, IgG1, and IgG2a were measured by ELISA. The 96-well flat-bottom microtiter plates (Sigma–Aldrich) were coated overnight at 4 °C with 100 μl of 100 μg/ml OVA in $NaHCO_3$ buffer (pH 9.6). Plates were washed three times with PBS–0.05% Tween 20, and then blocked by incubation with 200 μl 0.05% PBS–Tween and 1% BSA for 1 h at room temperature. After washing, different dilutions of serum samples and standard murine IgE, IgG1 and IgG2a (BD PharMingen, San Diego, CA) were added and the plates were incubated for 2 h at room temperature. OVA-specific immunoglobulins were detected with biotin-conjugated rat anti-mouse (1 μg/ml) IgE, IgG1, and IgG2a antibodies (BD PharMingen) by incubation for 2 h at room temperature. The plates were then washed and incubated with 1/1000 dilution of avidin–HRP-conjugated secondary antibody for 45 min at room temperature. The reaction was terminated with 2N H_2SO_4, and then read at 450 nm. Total IgE and IgG were measured and compared with know mouse IgE and IgG standards. Detection limit for total IgE and IgG was 100 pg/ml and 1 ng/ml, respectively.

2.9. Histological examination

Lungs were inflated and fixed in 10% neutral formalin. Paraffin sections (0.6 μm) were stained with Hematoxylin and Eosin (H&E), to examine tissue eosinophilia and Periodic-Acid Schiff (PAS), to examine goblet cell hyperplasia.

2.10. Statistical analysis

Data are expressed as means ± S.E.M. Levels of difference between all groups were determined by one-way analysis of variance followed by two-tailed Student's *t*-test, to determine the significant differences between pairs of groups. p-Values < 0.05 were considered significant.

3. Results

3.1. TQ inhibits PGD2, but not PGE2 production in the lungs of OVA-sensitized and challenged mice

TQ has been shown to inhibit arachidonic acid metabolism in cultured leukocytes. To determine whether TQ has an effect on PG biosynthesis in the asthma model used in this study, the pro-

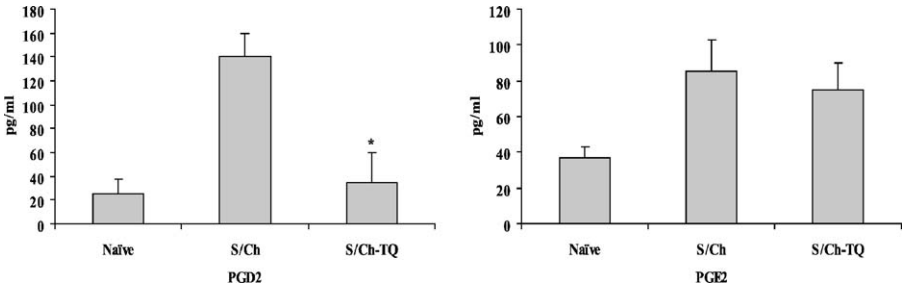

Fig. 1. TQ inhibits PGD2, but not PGE2, production in the airways. BAL fluid was collected 24 h after the last OVA challenge. Levels of PGD2 and PGE2 were measured using EIA and ELISA kit, respectively. Samples were applied in triplicate, and the assay was repeated three times, using BAL fluids from two separate experiments. Representative data (mean ± S.E.M.) from six mice per group are shown. * Significant difference compared with S/Ch group; $p < 0.05$.

duction of PGD2 and PGE2 was assessed in the BAL fluid. OVA sensitization and challenge resulted in an increased production of both proteins in the airways. Treatment with TQ resulted in a significant inhibition of PGD2, but with no effect on PGE2 (Fig. 1). This indicates that TQ has an effect on arachidonic acid metabolism and prostaglandin synthesis in vivo.

3.2. Effect of TQ on cyclooxygenase protein expression

Prostaglandins are products of arachidonic acid metabolism by cyclooxygenases (COXs). To determine whether TQ inhibited PGD2 biosynthesis by targeting cyclooxygenase expression, Western blotting was performed to examine the protein expression of the two cyclooxygenases (COX-1 and COX-2) in whole lung cells. The results (Fig. 2) showed that while COX-2 expression was induced significantly after OVA sensitization and challenge (S/Ch group), there was a slight induction of COX-1. When mice were treated with TQ before OVA challenge (S/Ch-TQ group), the increase in COX-1 and COX-2 expression was reduced to a level comparable to that seen in the Naïve mice, with more reduction in COX-2 level. However, there was no effect on COX-1 protein expression. In addition, administration of DMSO alone (compare Naïve and S/Ch groups) did not show any marked effects on COXs expression. These results show that TQ inhibits arachidonic acid metabolism and PGD2 synthesis by significantly inhibiting COX-2 expression that was induced by OVA antigen.

3.3. Effect of TQ on inflammatory cells and cytokine production in the airways

PGs are potent mediators of the Th2-biased inflammatory responses, including Th2 cells and cytokines. To determine whether the inhibition by TQ of COX-2 expression and PGD2 secretion observed above was associated with a change in the airway inflammation, we examined the accumulation of inflammatory cells and Th2 cytokine levels in the BAL fluid. OVA sensitization and challenge significantly increased the numbers

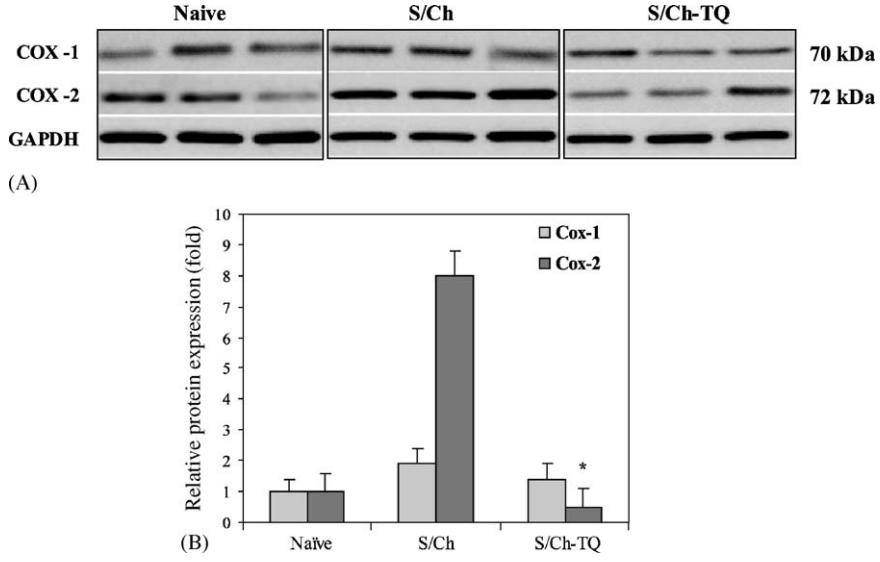

Fig. 2. Western blot analysis showing COX-1 and COX-2 protein expression in the lung. (A) Whole lung cells were isolated 24 h after the last OVA challenge. Cell lysates were run on 12% SDS-PAGE, and proteins were probed with anti-COX-1 and COX-2 antibodies. Results from three mice per group are shown. Data are representative of three separate experiments. (B) Densitometric analysis of Western blotting. Images were digitized using an Epson scanner (Long Beach, CA) and analysed using UN-SCAN IT digitizing software (Silk Scientific Inc., Orem, UT). Signal intensity for each band was normalized to GAPDH, and the numerical values were averaged for each group. The data represent the change in protein expression in treatment groups relative to the Naïve group, represented as one-fold. * Significant difference ($p < 0.05$) compared to Naïve group. Naïve, sensitized and challenged with saline and injected with DMSO alone; S/Ch, sensitized and challenged with OVA and injected with DMSO alone; S/Ch-TQ sensitized and challenged with OVA but administered with TQ in DMSO before the first OVA challenge.

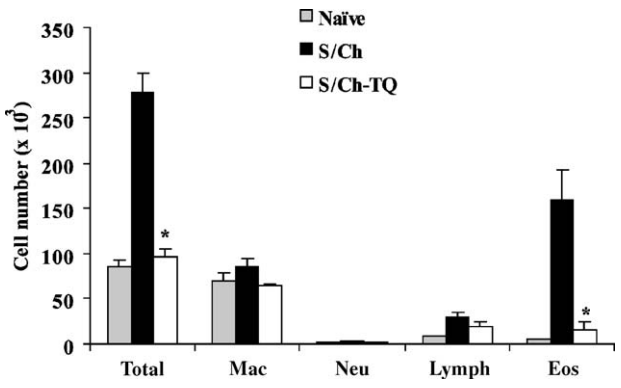

Fig. 3. TQ inhibits BAL cell inflammation in sensitized and challenged mice. Total and differential cell numbers were determined in BAL fluid obtained 24 h after the last OVA challenge in mice sensitized and challenged with saline and injected with DMSO alone (Naïve), mice sensitized and challenged with OVA and injected with DMSO alone (S/Ch), and mice sensitized and challenged with OVA and administered with TQ in DMSO (S/Ch-TQ). Data are expressed as mean ± S.E.M. of six mice per group and represent three separate experiments. Mac, macrophage; Neu, neutrophil; Lymph, lymphocyte; Eos, eosinophil. *Significant difference compared with S/Ch group; $p < 0.05$.

of inflammatory cells (Fig. 3) and the levels of IL-4, IL-5, and IL-13 cytokines in BAL fluid (Fig. 4). Eosinophils represented 57% of the total cell number in BAL fluid of sensitized and challenged mice. Treatment with TQ (S/Ch-TQ group) for 5 days before OVA challenge resulted in a significant ($p < 0.05$) decrease in the numbers of eosinophils in the airways (Fig. 3), and normalized the elevated levels of IL-5 and IL-13 to those seen in Naïve mice, whereas IL-4 was slightly higher in TQ treated mice compared with Naïve mice (Fig. 4). Also, numbers of lymphocytes were slightly reduced by TQ. Treatment with DMSO alone did not affect the inflammatory response (S/Ch group). Together, these data indicate that TQ suppresses airway inflammation by preventing the influx of eosinophils into the lungs and Th2 cytokine production in the airways of OVA-sensitized and challenged mice.

3.4. Effect of TQ on Th2 cytokine gene expression

The data presented in Fig. 4 showed that TQ significantly inhibited IL-4, IL-5, and IL-13 proteins secreted into the airways of OVA-sensitized and challenged mice. To assess whether TQ directly affect the cytokine response at the message level, total RNA extracted from whole lung cells was used in RT-PCR. The results (Fig. 5) showed that TQ normalized the increased levels of mRNA expression observed after OVA sensitization and challenge. The inhibitory effect of TQ on Th2 cytokine gene expression was almost parallel to the levels of the secreted proteins, indicating that TQ in DMSO, but not DMSO alone, affects Th2 cytokine responses at the level of gene expression.

3.5. Treatment with TQ attenuates lung tissue eosinophilia, mucus production, and allergen-specific immune response

To evaluate the effect of TQ on lung tissue inflammation, lung sections stained with H&E and PAS were examined for eosinophilic infiltrates into the lung tissue and mucus-producing goblet cells, respectively. OVA sensitization and challenge induced a massive infiltration of the peribronchial tissue with inflammatory cells (mainly eosinophils) (Fig. 6B), and goblet cell hyperplasia (Fig. 6E). TQ attenuated this inflammation by markedly reducing lung tissue eosinphilia and goblet cell hyperplasia (Fig. 6C and F), while DMSO alone had no effect on lung tissue inflammation (compare Naïve and S/Ch with S/Ch-TQ mice).

The effect of TQ on the humoral immune response to OVA sensitization and challenge was assessed by measuring the serum levels of OVA-specific antibodies (see Table 1). Sensitized and

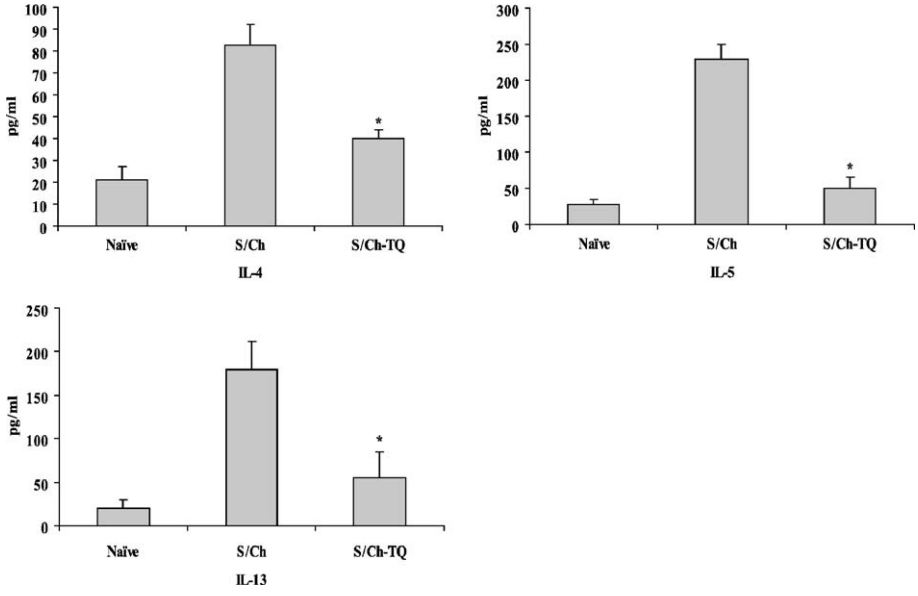

Fig. 4. Effect of TQ on Th2 cytokine profile of BAL fluid in sensitized and challenged mice. BAL fluid was collected 24 h after the last OVA challenge from the same groups described in the legend to Fig. 1. Levels of IL-4, IL-5, and IL-13 were measured (in triplicate) by ELISA. Mean ± S.E.M. of six mice per group representing data from three experiment are shown. *Significant difference compared with S/Ch group; $p < 0.05$.

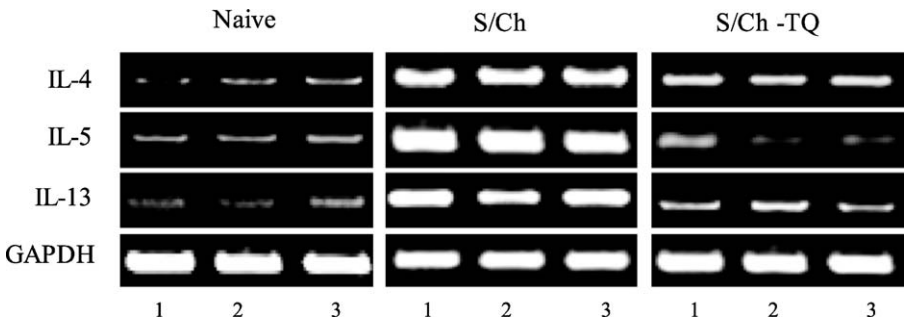

Fig. 5. Effect of TQ on Th2 cytokine gene expression. Whole lung cells were isolated 24 h after the last OVA challenge. Total RNA was extracted and used (1 μg) in RT-PCR to assess IL-4, IL-5, and IL-13 gene expression. GAPDH was amplified as an internal control. Results from three mice per each group are shown. Data are representative of two separate experiments. Naïve, sensitized and challenged with saline; S/Ch, sensitized and challenged with OVA; S/Ch-TQ, sensitized and challenged with OVA and administered with TQ before the first OVA challenge.

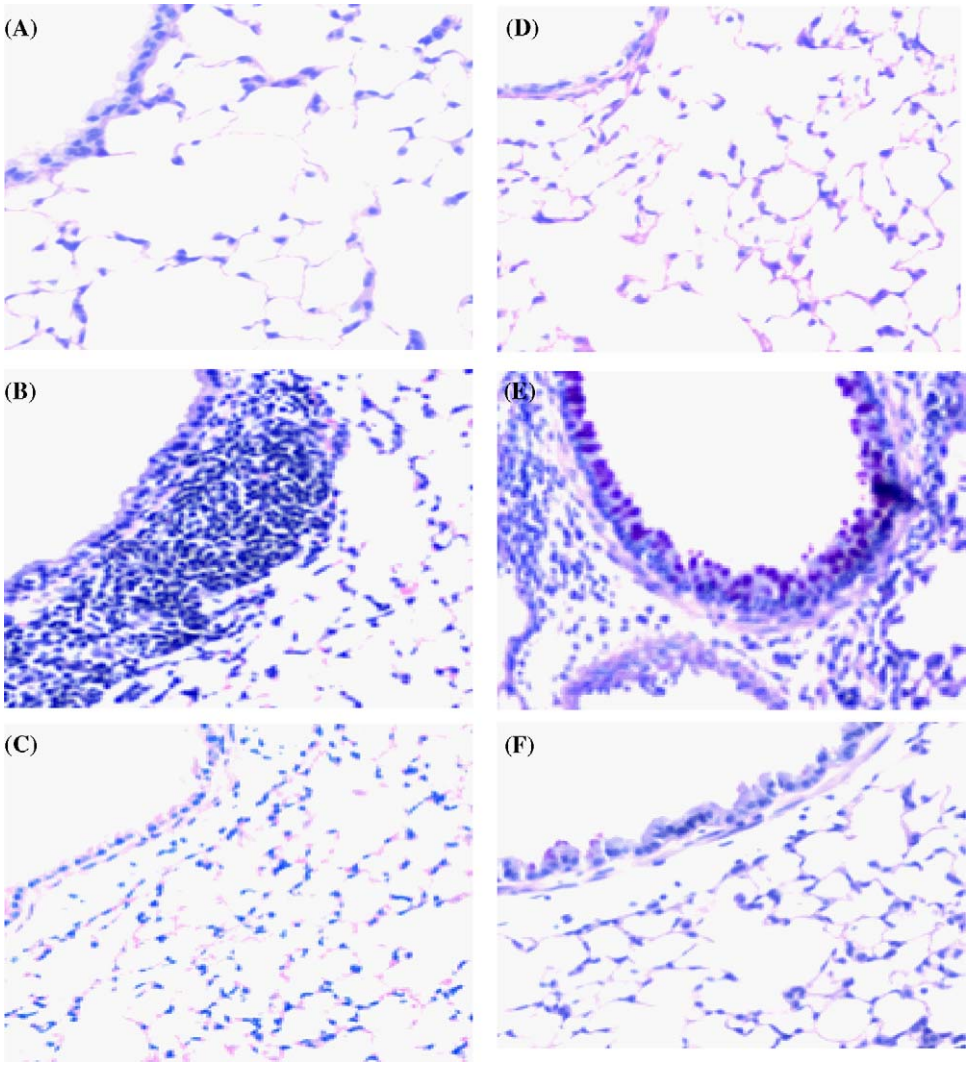

Fig. 6. Effect of TQ administration on lung tissue eosinophilia and goblet cell hyperplasia. Lung tissues from Naïve (A and D), S/Ch (B and E) and S/Ch-TQ (C and F) groups were processed 24 h after the last OVA challenge. Representative sections for each group are shown. Tissue eosinophilia was detected with H&E staining (A–C) and goblet cells were detected with PAS staining (D–F). Note the peribronchial eosinophil infiltration (B) and the hyperplasia of goblet cells (E) in the airways of sensitized and challenged mice (S/Ch group). Administration of TQ five times after sensitization but before OVA challenge markedly attenuated this inflammation (C and F).

Table 1
Treatment of mice with TQ decreases the elevated serum levels of OVA-specific Igs seen after sensitization and challenge

Group	Total (ng/ml)		OVA-specific (EU/ml)		
	IgE	IgG	IgE	IgGl	IgG2a
Naïve	44 ± 18	68 ± 24	<10	<10	<10
S/Ch	96 ± 34	112 ± 32	498 ± 86	866 ± 125	1076 ± 163
S/Ch-TQ	53 ± 20*	85 ± 29	39 ± 15*	45 ± 14*	284 ± 65*

Mice were sensitized, injected with TQ and challenged with OVA antigen as described in Section 2. Levels of serum immunoglubulins (Igs) were measured 48 h after the last OVA challenge. Data represent mean ± S.E.M. ($n = 6$), and represent two separate experiments. EU, ELISA unit; Naïve, sensitized and challenged with saline; S/Ch, sensitized and challenged with OVA; S/Ch, sensitized and challenged with OVA but received TQ for 5 days before the first OVA challenge.
* Significant difference ($p < 0.05$) compared with S/Ch group.

challenged mice developed significantly ($p < 0.05$) higher levels of total IgE and OVA-specific IgE, IgG1, and IgG2a, compared with the nonsensitized (Naïve) mice. Administration of TQ after OVA challenge resulted in a significant decrease in total IgE, OVA-specific IgE and IgG1, compared with S/Ch group, indicating that TQ inhibits the allergen-induced Th2-driven immune response.

4. Discussion

The volatile oil, and its main active constituent (TQ), extracted from *Nigella sativa* seeds, have been reported to exert anti-inflammatory effects on some diseases, including bronchial asthma [35–39]. Also, TQ has been shown to reduce proinflammatory lipid mediators by inhibiting eicosanoid generation in vitro [35]. Our results show that TQ attenuates OVA-induced airway inflammation by inhibiting COX-2 expression and PGD2 synthesis. This inhibitory effect on PG synthesis is accompanied by a significant decrease in Th2 cytokines, lung eosinophilia, goblet cell hyperplasia and Igs production in OVA-immunized and challenged mice.

Th2 cells play an important role in the pathogenesis of allergic airway inflammation through the production of Th2 cytokines [44–46]. These cells are recruited into the airway mucosa and their numbers are increased in the BAL fluid during the allergic asthmatic response [47–50]. Th2 cytokine levels, including IL-4, IL-5, and IL-13, are increased during the allergic response [51–54]. Our data showed that TQ lowered the elevated levels of IL-4, IL-5, and IL-13 protein production and mRNA expression that were seen in OVA-sensitized and challenged mice. IL-4 is required for Th2 cell differentiation and isotype switching in B cells from IgM to IgE [55,56]. It is also required for prostaglandins production by mast cells, and blocking IL-4 decreases serum IgE level and airway eosinophilia in allergic mice [57]. IL-5 and IL-13 regulate growth, differentiation, and survival of eosinophils [58–61]. In addition, IL-13 promotes IgE isotype switching in B cells and mucus production by goblet cells in the airway mucosa [62].

Eosinophil recruitment into the airways, and mucus hyperproduction by goblet cells are two key factors in the development and maintenance of the allergic inflammatory response [63,64]. IL-5 and IL-13 play an important role in eosinophil recruitment into the airways through inducing the expression of chemokines and adhesion molecules such as eotaxin and VCAM-1 [65–67]. On the other hand, IL-13 and IL-4 have been shown to increase mucus production in the airway epithelium [68,69], and to increase the levels of IgE and IgGs through inducing B cell growth and differentiation [70,62,54]. Our results showed that TQ significantly decreased the numbers of eosinophils in BAL fluid and lung tissue, the number of mucus-producing goblet cells in the airway mucosa, and the levels of OVA-specific IgE and IgGs. These effects were concomitant with the decrease in Th2 cytokines. Thus, these inhibitory effects by TQ on airway inflammation may be due to the decrease in Th2 cytokine levels.

PGD2 is the major eicosanoid product of arachidoinic acid metabolism by cyclooxygenase (COXs) and is produced by inflammatory cells including mast cells and Th2 cells in response to allergen [15,19–21]. Its levels are increased during the allergic asthmatic response [13,71]. Overproduction of PGD2 has been shown to exaggerate Th2 cytokine and eosinophilic inflammatory response in the airways of mice allergic to OVA [22]. In addition, mice deficient in PGD2 receptors (DPs) produce lower levels of Th2 cytokines, IL-4, IL-5, and IL-13, and reduced numbers of lymphocytes and eosinophils in the airways during an OVA-induced allergic response [27]. In contrast, a recent study investigated the role of CRTH2 (also known as DP2), a receptor for PGD2 expressed on Th2 cells and eosinophils that mediates chemotaxis, in a mouse model of allergic asthma reported that CRTH2 knockout enhanced eosinophil recruitment and airway inflammation [72]. Our data showed that PGD2 levels were increased in the airways after OVA sensitization and challenge, and that TQ administration strongly inhibited both PGD2 production and COX-2 expression, with only slight effects on COX-1. Since both COX-1 and COX-2 are upstream of PGD2 biosynthesis and drive the same pathway for arachidonic acid metabolism [11], we expected that in the absence of COX-2 expression, COX-1 might drive PGD2 synthesis, however, our data suggest that TQ may have an additional inhibitory effect on the PGD2 tissue-specific synthase, downstream of both COX-1 and COX-2.

Data regarding the role of cyclooxygenases and prostaglandins in allergic inflammation and asthma are somewhat controversial. While PGD2 and its receptors have been shown to exaggerate the inflammatory response by increasing Th2 cytokines and T lymphocytes and eosinophils in the airways of OVA-sensitized and challenged mice [73,22], inhibition of COX-1 and COX-2 have been shown to increase Th2 cytokines and airway eosinophilia in OVA-sensitized mice [73,74]. In addition, mice deficient in COX-1, but not COX-2, exhibit exaggerated levels of Th2 cytokines in response to OVA allergen [17]. Part of this controversy may be due to the use of different mouse strains, different experimental protocols, and applying physiological inhibitors versus primary gene deficiency of COX-1 and COX-2. Both enzymes are critical for T cell development [75] and their deficiency may affect the different aspects of the

immune and inflammatory response. Our data are in agreement with those of Honda et al. [76] and Fujitani et al. [22], which show an important role for PGD2 in enhancing the Th2-driven inflammatory response. In addition, a recent study [26] using two mouse models of allergic inflammation, reported that activation of the PGD2 receptor (DP2) exacerbates the inflammatory response, whereas activation of DP1 (a receptor for PGD2) ameliorates the pathogenesis. In another study, activation of CRTH2 (DP2) receptor by PGD2 was shown to restrict airway inflammation in asthma model by reducing IL-5 and eosinophils in the lung [72].

Finally, our data show that PGE2 was increased in the BAL fluid of OVA-sensitized and challenged mice, and that TQ did not affect PGE2 production significantly. PGE2 is present at high concentration in the lung [77] and play a protective role during the inflammatory response by inhibiting mast cell degranulation [78] and inflammatory cell recruitment into the airways [78,9]. In addition, inhalation of PGE2 decreases the number of eosinophils in the airway of asthmatic patients in response to allergen [9]. In our study, PGE2 production was increased after OVA sensitization and challenge and its level, as well as the level of COX-1 expression, was slightly reduced by TQ administration. Thus, it seems that PGE2 synthesis was driven through the basal levels of COX-1. The sustained production of PGE2 in the lung may enhance resolving airway inflammation induced by allergen exposure. It has been thought that the therapeutic and adverse effects of nonsteriodal anti-inflammatory drugs, which inhibit both COX-1 and COX-2, are mediated by the blocking of PGs synthesis. In addition, it has been suggested that the therapeutic and anti-inflammatory effect of these drugs is due to inhibition of COX-2 whereas their adverse effect is due to inhibition of COX-1 [79]. The exact mechanisms by which TQ inhibits allergic airway inflammation is not clear. Our data suggest that the anti-inflammatory effect of TQ is mediated, at least in part, by a mechanism that target COX-2 expression and PGs synthesis. TQ has also been shown to inhibit COX-1- and COX-2-catalyzed PGE2 biosynthesis in vitro, with more potent effect on COX-2 activity [80]. In addition, it has been reported that TQ inhibits inflammatory cell infiltration in the brain in an experimentally-induced encephalomyelitis in rats by a mechanism that involved the inhibition of NF-kB activation in the brain [81]. Also, TQ inhibited the inflammatory response in LPS-stimulated macrophages in vitro by blocking iNOS mRNA expression [82]. Our data clearly indicate that TQ is effective in preventing the allergic inflammatory response when given before the induction of airway inflammation with OVA challenge. Whether TQ has a therapeutic effect on allergic inflammation remains to be determined.

The current study reveals that the role of prostaglandins and cyclooxygenases in allergic inflammation is somewhat complex. We show that TQ suppresses the immunologic and inflammatory responses induced by PGs and Th2 cytokines in a mouse model of airway inflammation through the inhibition of COX-2 protein expression and PGD2 production in the lung. These findings support a potential role for the oil extract of *Nigella sativa* and its derived TQ in ameliorating allergic airway inflammation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.imlet.2006.04.012.

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