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Original article

The effect of Chinese herbal medicine, xiao-qing-long tang (XQLT), on allergen-induced bronchial inflammation in mite-sensitized mice

Background: There are detailed descriptions of the clinical experiences and prescriptions of asthma in traditional Chinese medicine. Xiao-qing-long tang (XQLT), or sho-seiryo-to by its Japanese name, is one of the Chinese herbal medicines used to treat bronchial asthma and allergic rhinitis for centuries. However, the therapeutic mechanisms of this medication are still far from clear. In this study, a house-dust-mite (*Dermatophagoides pteronyssinus* [Der p])-sensitized murine model of asthma was used to evaluate the immunomodulatory effect of XQLT on the allergen-induced airway inflammation in asthma.
Methods: Three different protocols were designed to evaluate the treatment and/or long-term prophylactic effect of XQLT in Der p-sensitized mice. XQLT extracts (1 gm/kg) were administered to sensitized mice 1 h before allergen challenge (AC) (group A), 24 h after AC (group B), and every other day six times before AC (group C), respectively. Cellular infiltration and T-cell subsets in the bronchoalveolar lavage fluid (BALF) of allergen-challenged mice were analyzed. Intrapulmonary lymphocytes were also isolated to evaluate their response to allergen stimulation.
Results: When XQLT was administered to the sensitized mice before AC (groups A and C), it suppressed airway inflammation by decreasing the number of total cells and eosinophil infiltration in the BALF, and downregulated the allergen- or mitogen-induced intrapulmonary lymphocyte response of sensitized mice as compared to those of controls. This immunomodulatory effect of XQLT may be exerted through the regulation of T-cell subsets by elevation or activation of the $CD8^+$ and double-negative T-cell population in the lung. However, the administration of XQLT to sensitized mice 24 h after AC (group B) did not have the same inhibitory effect on the airway inflammation as XQLT given before AC.
Conclusions: The administration of XQLT before AC has the immunomodulatory effect of reducing bronchial inflammation in the allergen-sensitized mice.

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Key words: bronchial asthma; Chinese herb medicine; xiao-qing-long tang.

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Allergic asthma is the most common chronic disease in children. The prevalence of this disease is increasing rapidly, and its seriousness has become greater in recent years. There have been numerous descriptions of the clinical experiences of and prescriptions for asthma in traditional Chinese medicine. However, the therapeutic mechanisms of these herbal medicines are unclear (1). Previously, an animal model of asthma was used to investigate the therapeutic effect of xiao-qing-long tang (XQLT), or sho-seiryo-to by its Japanese name, a traditional Chinese medicine used to treat bronchial asthma and allergic rhinitis for centuries (2, 3). The results showed that XQLT relieved the early and late asthmatic reactions, and reduced the local infiltration of inflammatory cells in the bronchoalveolar lavage fluid (BALF) of ovalbumin-sensitized guinea pigs. In this study, we plan to use our established murine model of asthma to study the immunomodulatory effect of XQLT on allergen-induced bronchial inflammation by analyzing the cellular distributions and T-cell subsets in the BALF of sensitized mice.

Material and methods

Reagents

Lyophilized house-dust mite (*Dermatophagoides pteronyssinus* [Der p]) was purchased from Allergon (Engelholm, Sweden). The crude mite preparation was extracted with ether. After dialysis with deionized water, the mite extract was lyophilized and stored at $-20^{\circ}C$ before use. Monoclonal antibodies used for fluorescence-activated cell-scanning (FACScan) staining of CD4 (clone H129.19), CD8 (clone 53–6.7), and

anti-CD3 (clone 145-2C11) were purchased from Pharmingen (San Diego, CA, USA). Mouse IgG1 and IgG2a conjugated with FITC or PE were purchased from Coulter Immunology (Hialeah, FL, USA) and used to determine the borderline between stained and unstained cells in the analysis of flow cytometry.

Preparation of extract of XQLT

Koda Pharmaceutics Ltd (Taoyuan, Taiwan) provided the medicinal plants used to prepare XQLT, or sho-seiryu-to. The preparation is a mixture of eight crude plant ingredients: Pinellaiae tuber (6.0 g, root of *Pinelia ternata* Breitenbach), Ephedrae herba (3.0 g, stem of *Ephedra sinica* Stapf), Schizandrae fructus (3.0 g, a fruit of *Schizandra chinensis* Baill.), Cinnamonomi cortex (3.0 g, cortex of *Cinnamomum cassia* Blume), Paeoniae radix (3.0 g, root of *Paeonia lactiflora* Pall.), Asari herba cum radice (3.0 g, whole plant of *Asiasarum heterotropoides* F. Maekawa var. *mandshuricum* F. Maekawa), Glycyrrhizae radix (2.0 g, root of *Glycyrrhiza uralensis* Fisch. Et. DC), and Zingiberis siccatum rhizoma (1.0 g, steamed root of *Zingiber officinale* Roscoe) (4). The XQLT was dissolved in distilled water for administration to mice. As for *in vitro* use, the water extract of XQLT was isolated by dissolving XQLT in distilled water, and then centrifuging the mixture at 7500 rpm for 30 min. After filtration, the aqueous extract was lyophilized and stored at -20°C. This extract of XQLT was dissolved in pyrogen-free isotonic saline (YF Chemical, Taipei, Taiwan) and filtered through a 0.2-μm filter (Microgen, Laguna Hills, CA, USA) before use.

Mice, immunization, and allergen challenge (AC)

Specific pathogen-free, 6–8-week-old BALB/c mice from the Laboratory Animal Center, National Cheng Kung University, were used in this study. The mice were housed in microisolator cages (Laboratory Products, Maywood, NJ, USA) and provided with sterile food and water *ad libitum*. The colony was monitored regularly for the presence of murine pathogens. Results were consistently negative. All experimental animal care and treatment followed the guidelines set up by the National Science Council of the Republic of China. Groups of mice were subcutaneously injected at the base of the tail with 50 μl of an emulsion containing 40 μg of Der p in complete Freund's adjuvant (CFA, Difco, Detroit, MI, USA) (5). Fourteen days later, the mice were lightly anesthetized with an intraperitoneal injection of 60 mg/kg of sodium pentobarbital (Nembutal, Abbott Laboratories, North Chicago IL, USA). They received intratracheal (i.t.) instillation with 50 μl of Der p (1 mg/ml) as allergen challenge (AC). The animals were held in an upright position for 1 min to resume normal breathing.

Experimental groups for oral administration of XQLT

Mice sensitized with Der p were divided into three different groups for XQLT administration. Group A was given 1 g of XQLT extract per kilogram of body weight (gm/kg) 1 h before Der p challenge; group B was given 1 g/kg XQLT 24 h after AC; and group C was given 1 g/kg of XQLT every other day six times, with the last treatment 48 h before AC. The control group was also sensitized with allergen but fed with PBS alone. Animals without sensitization, i.e., the naive group, or with sensitization but without XQLT treatment (the control group), were also included in the experiment for purposes of comparison. There were six mice in each group for every experimental condition.

Bronchoalveolar lavage fluid (BALF)

The mice were killed with an overdose of sodium pentobarbital at various intervals after challenge. BALF was collected with two separate 1-ml sterile endotoxin-free saline washes of lung via the trachea of each mouse. About 1.8 ml of the washing solution was consistently recovered. BALF cells were washed once with HBSS (Life Technologies, Grand Island, NY, USA) containing 2% fetal calf serum by centrifugation at 200 *g* at 4°C. Red blood cells in BALF were removed in lysing solution containing 20 mM NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA. After washing, the cells were resuspended in 1 ml of HBSS. The recovered BALF was pooled, passed through a double layer of gauze to remove gross mucus, and then centrifuged. Total leukocytes in BALF was determined with a hemocytometer, and the results were expressed as cell number per milliliter. The aliquot was then diluted to a concentration of 1×10^5 cells per milliliter, and 0.2 ml cell suspension was spun down onto a glass slide at 1100 rpm for 2 min with a cytocentrifuge (Cytospin 2, Shandon Instruments, Sewickley, PA, USA). The slides were then dried and stained by the May-Giemsa method. More than 200 cells were identified with a photomicroscope. All samples were evaluated in a double-blind manner. The remaining BALF cells were resuspended in RPMI-1640 (GIBCO/BRL, Life Technologies, Inc, Gaithersburg, MD, USA) with 10% heat-inactivated fetal bovine serum (GIBCO/BRL), and incubated in plastic dishes for 60 min at 37°C in 5% CO_2-humidified atmosphere to remove adherent macrophages that could interfere with accurate cell analysis. More than 90% of nonadherent cells collected for flow cytometric analysis were viable by the trypan blue exclusion test.

Flow cytometry analysis of lymphocytes in BALF

Specific binding of monoclonal antibody (mAb) was analyzed by direct immunofluorescence with a cyto-fluorograph (FACscan, Becton-Dickinson) by standard

methods recommended by the Becton-Dickinson Monoclonal Center (Mountain View, CA, USA). Briefly, 50 µl of cell suspension (1×10^5 cells) was incubated in the presence of saturating concentrations of fluoresein- or phycoerythrin-conjugated mAb in the dark on ice for 30 min. Erythrocytes were lysed by adding 3 ml lysing solution (0.155 mM NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM ethylenediamine tetraacetic acid (EDTA), pH 7.3, for 3–5 min. Leukocytes were washed twice with PBS containing 1% BSA and 0.1% sodium azide. Cytofluorometric analysis was performed with scatter gates set on the lymphocyte fraction by forward and side scatter (SCC) and PE fluorescence FL2 with laser excitation at 488 nm. The number of immunofluorescence-positive cells was determined out of 10 000 cells analyzed. Specific binding of mAb was controlled by subtraction of isotype-matched rat immunoglobulin. A computer system (Consort 30, Becton Dickinson) was used for data acquisition and analysis. List mode data for 5000 to 10 000 events were stored. A cell gate containing lymphocytes was established on the basis of forward and side light scatter. For determination of the borderline between stained and unstained cells, cells were also stained with mouse IgG1-conjugated FITC or PE. Percentages were calculated on the basis of the number of lymphocytes found in each quadrant.

Preparation of intrapulmonary lymphocytes

Mice were also killed by cervical dislocation. For each experiment, three to six mice were used, and the lung cells pooled for analysis, as described by Abraham et al. (6). In brief, the chest of the mouse was opened with two longitudinal incisions parallel to the sternum, avoiding the mammary vessels. The lung vascular bed was flushed by injecting 3–5 ml of chilled (4°C) BBS (balanced salt solution) without phenolphthalein into the right ventricle. The lungs were then excised, avoiding the peritracheal lymph nodes, and washed twice in BSS. The lungs were minced finely, and incubated in RPMI 1640 with 5% FCS, penicillin/streptomycin, 10 mM HEPES, 50 µM 2-ME, and 20 mM L-glutamine, containing 20 U/ml collagenase (C2139, Sigma Chemical Co., St Louis, MO, USA) and 1 µg/ml Dnase (type I, Sigma). A volume of 25 ml was used for four to six sets of lungs. After incubation for 60 min at 37°C on a rotary agitator (approximately 60 rpm), any remaining intact tissue was disrupted by passage through a 21-gauge needle. Tissue fragments and the majority of dead cells were removed by rapid infiltration through a glass-wool column, and the cells were collected by centrifugation. The cell pellet was suspended in 4 ml of 40% Percoll (Pharmacia, Uppsala, Sweden) and layered onto 4 ml of 80% Percoll. After centrifugation at 600 *g* for 20 min at 15°C, the cells at the interface were collected, washed twice in BSS, and counted. Viability as determined by trypan blue exclusion was consistently more than 98%. Cells to be used for the lymphocyte proliferation assay were plated in 24-well culture plates, and were allowed to adhere for 1 h at 37°C in a moist, 5% CO_2 incubator to remove macrophages. Nonadherent cells were collected and diluted into RPMI 1640, supplemented, as above, with HEPES, glutamine, penicillin/streptomycin, 2-ME, and 2% FCS.

Determination of allergen- and mitogen-induced lymphocyte proliferation by ^{3}H-labeled thymidine incorporation

Nonadherent intrapulmonary lymphocytes (5×10^4/ml) were incubated in 0.1 ml of the mentioned medium for assessment of proliferation by the incorporation of ^{3}H-labeled thymidine (20 µCi/mmol, New England Nuclear, Boston, MA, USA). For determination of the inhibitory effect of XQLT on allergen- or mitogen-induced lymphocyte proliferation, the extract of XQLT was first incubated with cells at various concentrations and incubation times, and then washed with medium five times to remove XQLT before allergen or mitogen (PHA or LPS) was added to induce cell proliferation. Allergen or mitogen solution was diluted in the same medium, and an aliquot of 0.1 ml was added to each well of a round-bottom, 96-well microtiter plate (Nunc, Denmark). Cells were incubated for 60 h at 37°C and 5% CO_2 incubator. For these experiments, 0.5 µCi of ^{3}H-labeled thymidine was added to each well, and incubation was continued for another 12 h. Cells were then harvested and repeatedly washed with a PhD cell harvester. Subsequently, incorporated thymidine was evaluated by measuring radioactivity in a liquid scintillation counter. All results are expressed as mean values in triplicate.

Statistical analysis

Results were all shown as mean ± SEM and analyzed either by Student's *t*-test or by analysis of variance. Comparison of group means was done with the Scheffes F test when all possible pairwise comparisons were made. Differences of $P < 0.05$ were judged to be significant. All experiments were repeated at least

Table 1. Bioactive components of XQLT

1. Herba Ephedrae (麻黃): 1-ephedrine, *d*-pseudoephedrine, 2,3,4,5,6-tetramethyl-pyrazine
2. Radix Paeoniae (芍藥): paeoniflorin, paeonol, benzoic acid, β-sitosterol
3. Herba Asari (細辛): methyleugenol, safrole, pinene, eucarvone, 1-asarinin
4. Rhizoma Zingiberis (乾薑): zingiberone, β-bisabolene, α-curcumene, β-sesquiphellandrene, zingiberol
5. Radix Glycyrrhizae (甘草): triterpene saponin, glycyrrhetic acid, flavones, licoricone
6. Ramulus Cinnamomi (桂枝): cinnamic aldehyde, trans-cinnamic acid, coumarin, β-sitos-terol protocatechuic acid
7. Fructus Schisandrae (五味子): schizandrin, deoxyschizandrin, gemisin, chamigrenal, chamigrene, sesquicarene
8. Rhizoma Pinelliae (半夏): β-sitosterol, β-sitosterol D-glucoside

Table 2. Changes of total cell counts and cellular distributions in BALF of naive mice 24 h after oral administration of XQLT

XQLT	Total cell counts ($\times 10^4$/ml)	Macrophages (%)	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)
0 g/kg	33.6 ± 2.2	23.4 ± 0.7 (94.7 ± 3.2%)	1.2 ± 0.4 (4.7 ± 1.7%)	0.2 ± 0.2 (0.7 ± 1.0%)	0
0.5 g/kg	22.0 ± 6.0	13.5 ± 2.5 (61.4 ± 10.1%)	7.2 ± 1.3 (33.4 ± 6.9%)	0.5 ± 0.2 (0.2 ± 0.1%)	0
1.0 g/kg	73.3 ± 4.1*	37.8 ± 7.8 (51.6 ± 10.7%)*	31.6 ± 3.6 (43.2 ± 4.9%)*	1.2 ± 0.9 (1.6 ± 1.2%)	0
4.0 g/kg	36.0 ± 9.4	14.8 ± 6.5 (41.1 ± 16.6%)*	12.8 ± 5.0 (35.6 ± 13.9%)*	0.9 ± 0.5 (2.7 ± 1.4%)	0

* $P < 0.05$, as compared to untreated (0 g/kg) mice.

twice. The data of study (with XQLT in different protocols) and control groups were analyzed and compared by one-way ANOVA.

Results

Effect of XQLT administration on cellular distribution in BALF of naive BALB/c mice

The individual bioactive components of XQLT are given in detail in Table 1. Previously, Minematsu et al. (7) have tested the oral single dose toxicity of XQLT on male and female Spraque-Dawley rats. The LD_{50} of XQLT was estimated to be greater than 8 g/kg. No observable abnormal clinical signs were attributable to the XQLT dosing, and there was neither loss of body weight nor grossly abnormal findings at autopsy. The changes of cellular distribution in BALF of naive mice at 24 h after treatment with various doses of XQLT extract are shown in Table 2. There was a marked increase in the percentage of lymphocytes and decrease of macrophages in the BALF after administration of 1 g/kg and 4 g/kg of XQLT, respectively. The total cell counts of BALF were also significantly increased in mice treated with 1 g/kg of XQLT as compared to those of untreated (0 g/kg) mice. We have found that the increased percentage of lymphocytes in the BALF of XQLT-treated naive mice was due to the increased subset of $CD3^+/CD8^+$ (60 ± 13% *vs* 23 ± 12%) and $CD4^-/CD8^-$ double-negative T cells (12 ± 5% *vs* 4 ± 2%) as compared to those of untreated mice. The change of cellular distribution in the BALF of naive mice treated by XQLT was dose- and time-dependent, as shown in Table 2 and Fig. 1. These cellular changes in BALF were most significant at 72 h when the mice were treated with 1 g/kg of XQLT, and gradually subsided to basal levels at day 7 after feeding (Fig. 1).

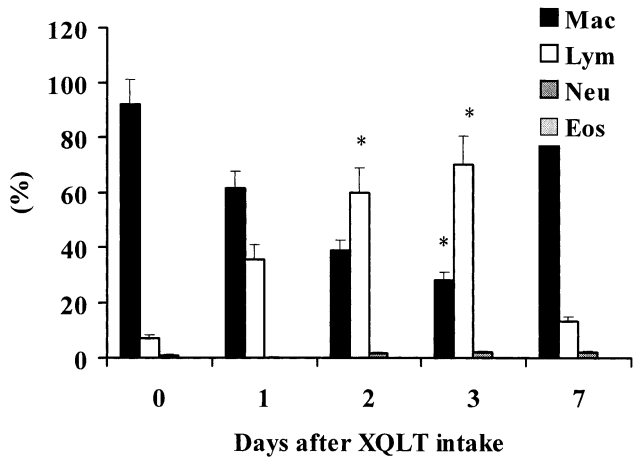


Figure 1. Change of cellular distributions in BALF of naive BALB/c mice after oral administration of 1 gm/kg of XQLT extract.

The prophylactic or therapeutic effect of XQLT on allergen-induced airway inflammation of sensitized mice

The changes of total cell counts and cellular distributions in BALF of groups of sensitized mice 72 h after allergen challenge are shown in Table 3. Intratracheal (i.t.) challenge of 50 μl Der p (1 mg/ml) of sensitized mice without XQLT treatment (controls) induced a marked increase in the number of infiltrated cells (Fig. 2a) and percentage of eosinophils in BALF (Fig. 2b) by 24 h after AC. Levels of the infiltrated cells peaked at day 3 and then gradually returned to baseline around day 7 after AC. The percentage change of eosinophils had the same pattern as total cellular infiltration in BALF that peaked at day 3, except that it remained high even at day 7 after AC. For evaluation of the prophylactic effect of XQLT on allergen-induced airway inflammation, sensitized mice were given XQLT (1 g/kg) 1 h before AC (group A), and the number of BALF cells and percentage of eosinophils were significantly lower than the PBS group at days 2, 3, and 7 after AC, respectively. Moreover, the percentage of lymphocytes and neutrophils (Table 3) also showed significant differences between group A and the control group (69.5% *vs* 31.2% in lymphocytes, and 20.5% *vs* 53.5% in neutrophils). There were also significantly lower numbers of infiltrated cells and percentage of eosinophils in the BALF at days 3 and 7 after AC of the sensitized mice that received six oral treatments of XQLT before AC (group C) as compared to those of control mice. Regarding the therapeutic effect of XQLT, there was no significant change in total cell infiltration and the percentage of eosinophils in the BALF of sensitized mice, when XQLT was administered 24 h after AC (group B), as compared to those in controls.

Table 3. Changes of total cell counts and cellular distributions in BALF of groups of sensitized mice 72 h after allergen challenge

Groups	Total cells (10^4 cells/ml)	Macrophages (%)	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)
Naive mice	41 ± 11	36.8 ± 5.3 (92.0 ± 11.2%)	2.8 ± 2.1 (7.0 ± 1.2%)	0.4 ± 0.1 (1.1 ± 0.3%)	0 (0%)
Controls	125 ± 23	41.1 ± 12.4 (33.7 ± 12.3%)	43.7 ± 24.1 (36.7 ± 12.9%)	18.1 ± 2.1 (17.0 ± 2.4%)	16.1 ± 3.5 (14.2 ± 3.2%)
Group A	79 ± 17	13.1 ± 2.1 (20.1 ± 9.3%)	45.4 ± 11.2* (69.5 ± 15.8%)	4.2 ± 2.4 (6.5 ± 3.1%)	2.8 ± 1.2* (4.0 ± 2.4%)
Group B	177 ± 37	11.9 ± 6.5 (10.7 ± 6.1%)	51.1 ± 21.9 (46.3 ± 13.2%)	18.3 ± 2.1 (18.0 ± 2.3%)	28.7 ± 6.3 (25.1 ± 6.5%)
Group C	64 ± 29	12.6 ± 2.1 (22.5 ± 4.5%)	37.0 ± 11.3* (66.2 ± 16.3%)	3.4 ± 1.2 (6.2 ± 2.1%)	3.1 ± 1.2* (5.5 ± 2.1%)

* $P < 0.05$, as compared with PBS-treated sensitized (controls) mice.

Flow cytometry analysis of T-cell population in the BALF of sensitized mice

The effect of XQLT on the percentage change of T-cell subsets was determined by flow cytometry with immunofluorescence of monoclonal antibodies to direct staining of CD3, CD4, and CD8 molecular on infiltrated lymphocytes in the BALF of sensitized mice (Fig. 3). There was significant decrease in the percentage of $CD3^+/CD4^+$ lymphocytes in groups A and C mice as compared to those of control mice, respectively. Moreover, the percentage of double-negative ($CD4^-/CD8^-$) T cells in the BALF of sensitized mice was also increased when mice were treated with single as well as multiple doses of XQLT before AC. The percentage of $CD3^+/CD8^+$ lymphocytes was increased in groups A and C, but not to a statistically significant extent as compared to controls. The change of T-cell subsets in the BALF of group B mice was not significantly different from those of controls.

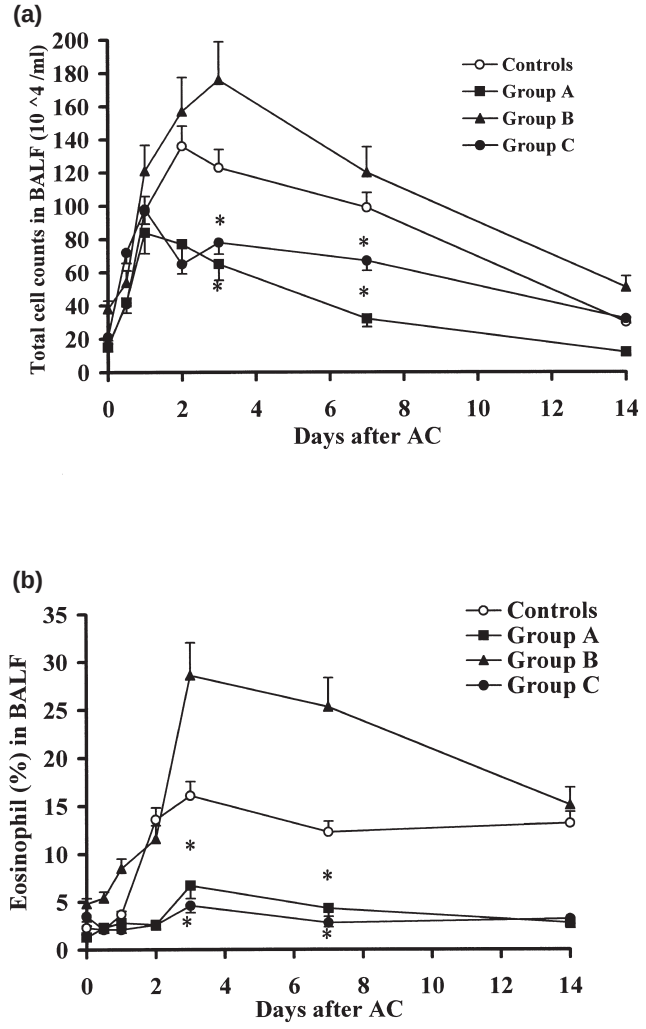


Figure 2. Changes of total cell counts (a) and percentage of eosinophils (b) in BALF of sensitized mice after allergen challenge with different treatments of XQLT or PBS. *$P < 0.05$, as compared with PBS treated (controls) sensitized mice.

Effect of XQLT on the allergen- or mitogen-induced intrapulmonary lymphocyte proliferation

We have also isolated intrapulmonary lymphocytes from sensitized mice at day 3 after AC to assay the inhibitory effect of XQLT on allergen- or mitogen-induced lymphocyte proliferation. The results in Fig. 4 show that there was dose-dependent inhibition of XQLT on PHA-, LPS- or Der p-allergen-stimulated lymphocyte proliferation in the sensitized mice. XQLT had a significant suppressive effect on Der p-stimulated lymphocyte proliferation at the concentrations of 10 μg/ml, as compared to Der p-stimulated cells alone ($P < 0.05$). Similarly, PHA- or LPS- stimulated lymphocyte proliferation was also suppressed in the

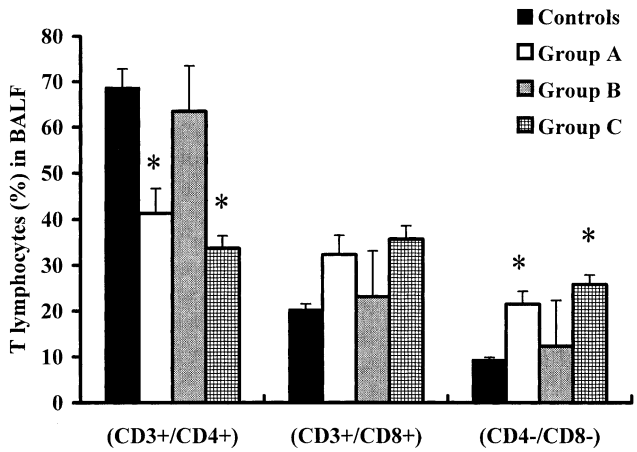


Figure 3. Effect of XQLT on T-cell subsets in BALF of sensitized mice 72 h after AC. *$P < 0.05$, compared with PBS group.

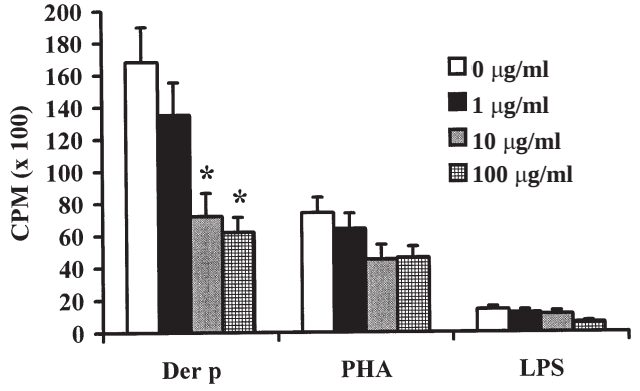


Figure 4. Effect of XQLT on allergen- or mitogen-induced intrapulmonary lymphoproliferation. $*P<0.05$, as compared with mitogen-stimulated lymphocytes without XQLT.

presence of XQLT extract, but was not statistically significantly different from that of mitogen-stimulated lymphocytes without XQLT.

Discussion

We have recently demonstrated that allergens of house-dust mites may induce eosinophilia in sensitized mice (5, 8). Such eosinophilia was $CD4^+$ T-cell dependent and associated with a series of inflammatory and immunologic events, including the development of early-type hypersensitivity, tumor necrosis factor (TNF)-α production, VCAM-1 expression, and tracheal hyperreactivity. In the present study, a murine model of asthma, prepared and characterized as described, was designed to evaluate the prophylactic and/or therapeutic effect of the Chinese herbal medicine XQLT on allergen-induced lymphocyte proliferation and airway inflammation in dust-mite-allergen (Der p)-sensitized BALB/c mice. We have shown that there was significant decrease in the amount of cellular infiltration and percentage of eosinophils in the BALF of sensitized mice that had been treated with single and multiple doses of XQLT before AC. Moreover, the percentage of lymphocytes in the BALF was significantly increased in group A and group C as compared to that of the control group. Flow cytometry analysis has shown that this increase of lymphocyte percentage in BALF was mainly due to increase in the percentage of the $CD8^+$ T-cell subset and, to a minor degree, to double-negative ($CD3^+$/$CD4^-$/$CD8^-$) T cells.

T cells of a Th2-like phenotype (9, 10) are thought to play an important role in orchestrating the asthmatic inflammatory response. When atopic asthmatics are exposed to allergen, it has been found that $CD4^+$ T cells are depleted in the circulation and sequestered in the lung (10). Moreover, an elevation in double-negative T cells was observed in subjects who develop isolated early asthmatic response, but not late-phase response. It is proposed that the function of $CD4^-$/$CD8^-$ cells is to downregulate or suppress the late inflammatory response (11). Therefore, the immunomodulatory role of XQLT in the allergen-sensitized mice may lie in regulation of T-cell activation. Further studies are needed to verify the effect of XQLT in changing T-cell subsets during allergic inflammation.

Previous reports showed that administration to mice of 8 gm/kg of XQLT daily would induce a high energy state, and cause no harm to the liver, as shown by autopsy (7). In order to determine whether there was any therapeutic and/or long-term prophylactic effect of XQLT on allergen-induced airway inflammation, we used three different protocols in administration of 1 gm/kg of XQLT extracts to sensitized mice 1 h before AC (group A), 24 h after AC (group B), and every other day six times before AC (group C). We found that group A and group C showed the same prophylactic effect of reduced cellular infiltration and decreased eosinophil percentage in the BALF during allergen-induced bronchial inflammation. However, at 24 h after AC and at the start of the late phase of allergic inflammation, the administration of XQLT did not significantly suppress the cellular infiltration in the BALF of group B sensitized mice as compared to those of the control group. Moreover, the percentage of eosinophils in the BALF of group B mice was markedly increased when they were treated with XQLT after AC. We are still unable to explain the different response of these two protocols in sensitized mice. It may be due to the allergen-induced bronchial inflammatory process in the sensitized mice, and the fact that the XQLT treatment did not only lose its suppressive effect, but it also enhanced the already activated CD4 T-cell function of recruiting more eosinophils and worsening the inflammation of the lung. Of course, this conception of the XQLT pharmacologic function must be confirmed by further study.

There are only a few publications on the immunomodulatory effect of XQLT, and its mechanism is still far from clear (2–4, 12–15). Eda (2) reported that XQLT did not have significant suppressive effects on IL-2 production and IL-2 receptor expression on PBMC in intractable asthma stimulated by *Candida*. But XQLT did have suppressive effects on both LTC_4 and O_2^- production (3, 13). These results suggest that XQLT inhibited production of LTC_4 and O_2^- by neutrophils, preventing prolonged bronchoconstriction and irreversible changes in small airways and allergic rhinitis (2, 15). Tanno et al. (12) used the conditioned medium of the T-cell leukemia cell line Mo as a growth factor to investigate the *in vitro* effect of XQLT on human basophil growth. They found that XQLT, as well as other antiallergic agents such as ketotifen, can inhibit the growth of human basophils and histamine release as detected in culture supernatant. In another study comparing the different bioactivities of various kinds of Chinese herbal medicines, as opposed to the other

medicines tested in the study, XQLT showed little effect of increased synthesis of G-CSF by PBMC from normal volunteers (13). In a preliminary study, we have also found that XQLT inhibited LTB_4 production, but had no effect on histamine release by peripheral blood mononuclear cells from Der p-sensitized asthmatic children (unpublished data). Therefore, the different responses between group A (prophylactic) and group B (therapeutic) may be due to the anti-inflammatory effect of XQLT on the different phases of allergen-induced airway inflammation.

In summary, we found that water extract of XQLT administered to allergen-sensitized mice 1 h before allergen challenge and every other day six times before AC suppressed airway inflammation by decreasing the amount of cellular infiltration and percentage of eosinophils in the BALF, and downregulating the allergen- or mitogen-induced intrapulmonary lymphocyte response. This immunomodulatory effect of XQLT may be exerted through regulation of T-cell subsets by elevation or activation of the $CD8^+$ and double-negative T-cell population. However, the administration of XQLT to sensitized mice 24 h after AC did not have the same inhibitory effect on the airway inflammation as XQLT given before AC. The mechanism of these two different responses of XQLT remains unclear. Further studies are needed to determine the precise role of this herbal medicine in the treatment of asthma.

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