編號：**CCMP87-RD-033**

小青龍湯對於過敏氣喘動物之免疫調控的影響

The Immunomodulate Effect of Xiao-Qing-Long-Tong on Mite-sensitioe Allergic Mice

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摘要

過敏性氣喘是在兒科領域中最常見的慢性疾病，而且其盛行率有愈來愈增加的趨勢，對於氣喘產生的因素至今仍未清楚，而現代醫學的藥方仍侷促在症狀緩解及抗發炎的類固醇的使用。由於在中醫古典醫籍中，已累積了豐富的臨床經驗與治療方劑，許多治療機轉仍付之闕如，因此我們利用過去已建立好的過敏氣喘的動物模式，來探討小青龍湯的致病機轉。

由於過去研究中顯示，小青龍湯能有效治療氣喘發作所引起的立即與遲發性過敏發炎反應，並能減少局部發炎細胞的浸潤，所以我們假設小青龍湯除了直接鬆弛呼吸道平滑肌外，可能也有使過敏免疫發炎反應的TH2反應逆轉成正常的TH1反應，因此針對小青龍湯可能引起的免疫調控作用，我們進行了下列實驗。我們將BALB/C的老鼠，以過敏原塵蟎注射至老鼠尾部皮內形成致敏反應，再過七天後以同樣劑量的過敏原再追加注射一次後，再等一星期後進行氣管內過敏原刺激誘發試驗，我們除了以PBS做為對照餵食外，將致敏老鼠分成三組，組(A)是在誘發試驗前餵食小青龍湯，組(B)是在誘發試驗後24小時再給一次小青龍湯。組(C)是在起始致敏後，每隔一天即餵食小青龍湯共六次，最後一次是在誘發試驗四十八小時前。在

進行完這些實驗的情形況後，我們觀察實驗與對照組老鼠的氣管肺泡沖洗液，並分析其發炎細胞的組成，T淋巴球的族群，血中單核球對過敏原刺激的增生反應及血清中IgE過敏抗體的高低值。我們發現組(A)的老鼠在誘發試驗前餵食小青龍湯，可以改變肺泡沖洗液內各個發炎細胞的組成(即吞噬中性球減少淋巴球細胞增加)，恰與對照組相反，尤其是引起過敏氣喘的主要發炎細胞—嗜伊紅球細胞有顯著的下降。而相反的組(B)的老鼠在誘發試驗後24小時再餵食小青龍湯，恰使得氣喘發炎情況更惡化。而組(C)也有類似組(A)的改善情形產生，我們進一步分析T淋巴球的組成發現，組(A)的老鼠CD4+與CD4- ,CD8-兩組T淋巴球次群有明顯的增加，這也許可解釋小青龍湯的免疫調控反應，不過需進一步的實驗方能證實。我們的初步結論，是小青龍湯在已致敏但尚未誘發氣喘的老鼠中可以有免疫調控作用，而減少或預防隨後而來的氣喘發炎反應。但若已在氣喘發炎反應已形成後，反而會惡化病症增加沖洗液內之發炎細胞。此一現象是否與小青龍的中藥醫理或免疫調控作用有關，需待進一步實驗探索方知。

關鍵詞：小青龍湯、氣喘動物、過敏免疫調控

ABSTRACT

Allergic asthma is the most common chronic disease in children. The prevalence of this disease is increased rapidly in recent ten years and the cause of increased incidence is unknown either. There were detailed descriptions about the pathologic mechanisms, symptoms, clinical experiences and therapeutic prescriptions of asthma in the traditional Chinese medicine. However, many therapeutic mechanisms are still unclear. Previously, there were studies that used animal model to investigate the therapeutic mechanisms of Xiao-Qing-Long-Tang which also frequently used in clinics. The results showed that Xiao-Qing-Long-

Tang (XQLT) was useful to relieve the early and late asthmatic reactions and reduce the local infiltration of inflammatory cells. This study was proceeded according to the methods of our previous reports. We found that chronic use of XQLT before allergen challenged can inhibit the allergen-induced bronchial inflammation, while after allergen challenge, XQLT will exert harmful effect by aggravate eosinophils infiltration and bronchial inflammation. There were significant decrease percentages of eosinophils and macrophages, and increase of lymphocytes population in BAL of group A (feeding XQLT 30 min before allergen challenge) as compared to those of PBS group. While the cellular distribution in BAL of group B (feeding XQLT 24 hr after AC) and group C (long term use of XQLT) had no significant change as compared to that of PBS group. BAL cells from allergen-challenged mice were also assessed with intracellular staining by FAScan. Mononuclear cells obtained from peripheral blood and peribronchial lymph node were analyzed for the response to XQLT *in vitro*. There was significant increase in the percentage of CD3+/CD4+/CD8- lymphocytes in the group A mice as compared to those of PBS group mice. Moreover, the percentage of double negative (CD4-/CD8-) T lymphocytes in the BAL of sensitized mice were also increased when treated with XQLT 30 min before AC. We suggested that the therapeutic mechanisms of Xiao-Qing-Long-Tang were through change of Th2/Th1 cytokine profile that induced immunological tolerance in the airway and reduced some degree of allergic inflammation. Therefore, in the future study, we plan to study firstly the immunomodulatory effect of XQLT on the allergen-induced bronchial inflammation after long term used of this herb medication.

Key words: Xiao-Qing-Long Tong, bronchial asthma, immunomodulatory effect

(1) Introduction:

Allergic asthma is a common disease. The prevalence of this disease increased rapidly and its seriousness becomes worse and worse in recent years. There were detailed descriptions about the pathologic mechanisms, symptoms, clinical experiences and therapeutic prescriptions of asthma in the books of traditional Chinese medicine. However, many therapeutic mechanisms are still unclear. The asthmatic animal model was used in this study to investigate the therapeutic mechanisms of Xiao-Qing-Long-Tang that was used often in clinics. Previously results showed that Xiao-Qing-Long-Tang was used to relieve the early and late asthmatic reactions and reduce the local infiltration of inflammatory cells. Therefore, in this study, we plan to use our previously established allergen-sensitized murine model of asthma to study the effect of XQLT on the allergen-induced bronchial inflammation, and to study the immunomodulatory effect of XQLT by analyzed the cellular distributions and T lymphocyte subsets in the bronchoalveolar lavage fluid from sensitized and allergen challenged mice.

(2) Materials 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 °C before use. Monoclonal antibodies used for fluorescence-activated cell scanning (FACS) staining against CD4 (clone H129.19), and CD8 (clone 53-6.7), anti-CD3 (clone 145-2C11) were purchased from Pharmingen (San Diego, CA, USA). Mouse IgG_1 and IgG_{2a} conjugated with FITC or PE were purchased from Coulter Immunology (Hialeah, Fl.) and used to determine the borderline between stained and unstained cells

in the flow cytometric analysis.

Total and Specific IgE Ab concentration in serum. The concentrations of total IgE Ab and Specific IgE Ab against Der p were detected by the methods previously described [Yu et al]. In brief, total IgE Ab was determined by a comercial IgE Kits (PharMingen). The specific IgE agiant Der p was

Preparation of extract of Xiao-Qing-Long Tang (XQLT). Medicine plants used for preparation of *Xiao-Qing-Long Tong* (X.Q.L.T; or Sho-seiryu-to) were provided by Koda Pharmaceutic Co. LTD, (Taoyuan, Taiwan). The preparation is an mixture of eight kinds of crude drugs consisting of Pinellaiae Tuber (6.0g, root of *Pinelia ternata* Breitenbach), Ephedrae Herba (3.0 g, stem of *Ephedra sinica* Stapf), Schizandrae Fructus (3.0h, fruit of *Schizandra chinensis* Baill.), Cinnamonomi Cortex (3.0 g, cortex of *Cinnamonmum cassaia* 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 (2.0g, root of *Glycyrrhiza uralensis* Fisch. Et DC) and Zingiberis Siccatum Rhizoma (1.0 g, steamed root of *Zingiber officinale* Roscoe). [Nagai et al] The XQLT was dissolved in distilled water to administrated to mice. As for *in vitro* used, the water extract of XQLT was isolated by dissolved XQLT in distill water then centrifuged 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 0.2 μm filter (Microgen, Laguna Hills, Calif., USA) before use.

Mice, immunization and challenge. Specific pathogen-free, 6-8-week-old BALB/c mice from the Laboratory Animal Center, National Cheng Kung University, were used in this study. Mice were housed in Micro-isolator cages (Laboratory products, Maywood, N.J., 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 ul of an emulsion containing 40 ug of Der p in CFA (Difco, Detroit, Mich., USA) [Yu et al]. Fourteen days later, mice were lightly anesthetized with intraperitoneal injection of 60 mg/kg of sodium pentobarbital (Nembutal, Abbott Laboratories, North Chicago ILL., USA). The mice were received intra-tracheal (I.T.) instillation with 50 μl of Der p (1 mg/ml) allergen challenge (AC). 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 in two different treatments of XQLT administration. Group A was given 1g/ml/kg of XQLT extract 30 min before I.T. Der p challenge, Group B was given 1g/ml/kg 24 hr after I.T. allergen challenge. Group C was given 1 g/ml/kg of XQLT every other day for 6 times with the last treatment on 48 hr before AC. PBS group was fed with PBS alone. Several animals with or without immunization were also used for reaction-negative controls in the experiment.

Bronchoalveolar lavage (BAL) fluids. Mice were euthanized with an overdose of sodium pentobarbital at various intervals after challenge. BAL fluids were collected with two separate 1-ml sterile endotoxin-free saline washes of lung via the tracheal of each mouse. About 1.8 ml of the washing solution were consistently recovered. BAL cells were washed once with HBSS (Life Technologies, Grand Island, N.Y., USA) containing 2 % fetal calf serum by centrifugation at 200 g and at 4 °C. Red blood cells in BAL fluids 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 BAL was pooled, passed through a double layer of gauze to remove gross mucus, then centrifuged. Total leukocytes in BAL fluids were 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 1,100 rpm for 2 min using a cytocentrifuge (Cytospin 2; Shandon Instruments; Sewickley, Pa). The slides were then dried and stained using the May-Giemsa method. More than 200 cells were identified using a photomicroscope. All samples were evaluated in a double-blind manner. The remaining BAL cells were resuspended in RPMI-1640 (GIBCO/BRL; Life Technologies Inc; Gaithersburg, MD) 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 BAL fluid. Specific binding of monoclonal antibody (MoAb) was analyzed by direct immunofluorescence according to standard methods recommended by Becton-Dickinson Monoclonal Center (Mountain View, CA) using a cytofluorograph (FACscan, Beckton-Dickinson). Briefly, 50 µl of cell suspension (1×10^5 cells) was incubated in the presence of saturating concentrations of fluoresein-or phycoerythrin-conjugated MoAb in the dark on ice for 30 min. Erythrocytes were lysed by adding 3 ml lysing solution (0.155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM ethylenediamine tetra-acetic acid (EDTA), pH 7.3) for 3-5 min. Leukocytes were washed twice with PBS containing 1 5BSA and 0.1% sodium azide. Cytofluorimetric analysis was performed with scatter gates set on the lymphocyte fraction by forward and side scatter (SCC) and PE fluorescence FL2 using laser excitation at 488 nm. The number of immunofluorescence-positive cells was determined out of 10,000 cells analyzed. Specific binding of MoAbs 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 5,000 to 10,000 events were stored. A cell gate containing lymphocytes was established on the basis of forward and side light scatter. To determine 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 [Abraham et al]. In brief, the chest of the mouse was opened using two longitudinal incisions parallel to the sternum, which avoided the mammary vessels. The lung vascular bed was flushed by injecting 3 to 5 ml of chilled (4 °C) BBS (balanced salt solution) without phenolphthalein into the right ventricle. The lungs were then excised, avoiding the peri-tracheal 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, 20 mM L-glutamine, containing 20 U/ml collagenase (C2139, Sigma Chemical Co., St Louis, MO) and 1 μg/ml Dnase (type I, Sigma). A volume of 25 ml was used for four to sex 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 cells 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 x *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 lymphocyte proliferation assay were plated in 24-well culture plates, and were allowed to adhere

for 1h at 37 °C in a moist, 5% CO2 incubator to removed macrophages. Non-adherent 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] thymidine incorporation. 5 x 10^4 non-adherent intrapulmonary lymphocytes were incubated in 0.1 ml above medium for assessing proliferation involved the incorporation of [^{3}H] thymidine (20 Ci/mmol, New England Nuclear, Boston, MA). To determine the inhibitory effects of XQLT on allergen- or mitogen-induced lymphocytes proliferation response, the extract of XQLT was first incubated with cells, respectively, at various concentrations and incubation time, and then washed with medium for 5 times to remove XQLT before allergen or mitogen (PHA or LPS) was added to induce cells proliferation. Der p or PHA or LPS solutions were 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] thymidine was added to each well, and incubation continued for another 12 h. Cells were then harvested and repeatedly washed using a PhD cell harvester. Subsequently, incorporated thymidine was evaluated by measuring radioactivity in a liquid scintillation counter. All results are expressed as mean values of triplicated.

Statistical analysis. Results were analyzed either by Students test or by analysis of variance. Comparison of group means was done with Scheffes F test when all possible pair-wise comparison were made. Differences with $p < 0.05$ were judged significant. All experiments were repeated at least twice. The data of study (with Xiao-Qing-Long-Tang) and control groups were analyzed for Th2/Th1 cytokine profile change and compared data by one-way ANOVA.

(3) Results

1. Effect of XQLT feeding on total cell numbers and cellular distribution in bronchoalbeolar lavage fluid from naïve BALB/c mice.

XQLT is Chinese tradition medicine that prepared from an extarct of a mixture of eight herb drugs, i.e. 6 parts of Pinelliae Tuber, 3 parts of Glycyrrhizae Radix, 3 parts of Cinnamomi Cortex, 3 parts of Schisandrae Fructus, 3 parts of Asiasari Radix, 3 parts of Paeoniae Radix, 3parts of Ephedrae Herba and 3 parts of Zingiberis Siccatus Rhizoma. A single oral dose toxicity study of XQLT was performed on male and female Spraque-Dawley (SD) rats. XQLTs LD50 was estimated to be greater than 8 g/kg. No observable abnormal clinical signs were attributable to the XQLT dosing, and neither body weight nor grossly abnormal finding in autopsis. [Minematsu et al]. Therefore, we have test the effects of single oral intake of low dose (0.2g/kg) and high dose (1 g/kg) on the cellular change of BAL of naïve BALB/c mice. The results showed that there is marked increase percentage of lymphocyte and decrease of macrophages in BAL fluid after 24 hr fed with XQLT, despite no significant change of total cells count before and after feeding. This phenomenon of cellular percentage change was dose-dependent and gradually back to original at 7 days after feeding.

2. Effects of XQLT on total cell numbers and percentage of eosinophils in bronchoalveolar lavage (BAL) after allergen (Der p) challenge (AC).

Intratracheal (I.T.) challenge of 50 μg crude tract of Der p to sensitized BALB/c mice that fed on PBS (PBS group) induced a marked increase in the number of BAL cells (Fig. 1) and percentage of eosinophils (Fig. 2) 24 h after AC. The BAL cells peaked at day 3 and then gradually returned to baseline around day 7 AC. The percentage change of eosinophils has the same pattern as numbers of total cells in

BAL that peaked at day 3, except it remained high even after 7 days after challenge. When sensitized mice were given XQLT (1g/kg) 30 min before AC (Group A), the number of BAL cells and percentage of eosinophils were significantly lower than PBS group at day 2, 3, and 7 AC, respectively. Moreover, the percentage of lymphocyte and neutrophils also showed significantly change between Group A and PBS control group (69.5% vs 31.2 % in lymphocyte; 20.5 % vs. 53.5 % in neutrophils). On the contrary, if the XQLT was administrated 24 hr after AC (Group B), there is increased total cells counts and the percentage of eosinophils in the BAL as compared to those in PBS group. There were also significantly lower numbers of BAL cells at day 3 AC in the sensitized mice taken 6 times of oral treatment of XQLT before AC (group C) as compared to those of PBS group. While the percentage of eosinophils of BAL cells remain high, and make no difference as compared to that of PBS group.

3. Effects of XQLT on cellular distribution in BAL after Der p challenge (AC).

In Fig. 3, results of cellular distribution in BAL of different groups were compared at day 3 AC. There were significant decrease percentages of eosinophils and macrophages, and increase of lymphocytes population in BAL of group A as compared to those of PBS group. While the cellular distribution in BAL of group B and group C had no significant change as compared to that of PBS group.

4. Flow cytometry analysis of T-lymphocyte population in BAL.

The total number of lymphocyte population in BAL fluid was increased significantly at days 3 in the group A mice as compared to that of PBS group (Fig. 3). Therefore, flow cytometry analysis of T-lymphocyte subsets percentage was performed by using immunofloresence monoclonal antibodies to direct staining of CD3, CD4, and CD8 molecular on lymphocytes in the BAL (Fig. 4). There is

significant increase in the percentage of CD3+/CD4+/CD8- lymphocytes in the group A mice as compared to those of PBS group mice. Moreover, the percentage of double negative (CD4-/CD8-) T lymphocytes in the BAL of sensitized mice were also increased when treated with XQLT 30 min before AC.

5. Effect of XQLT on the allergen- or mitogen-induced intra-pulmonary lymphocyte proliferation.

We have also isolated intra-pulmonary lymphocytes from sensitized mice at day 3 after AC to assay the inhibitory effect of XQLT on the allergen- or mitogen-induce lymphocytes proliferation. Results in Fig. 5. 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 presence of XQLT extract, although there were no statistically significant inhibition as compared to the mitogen-stimulated lymphocytes alone.

(4) Discussion

We have recently demonstrated that house dust mite (HDM) allergen could induce eosinophilia in sensitized mice [9, 12]. The HDM-induced eosinophilia was CD4+ T cell dependent and associated with a series of inflammatory and immunological events including the development of early-type hypersensitivity, tumor necrosis factor-(TNF)-_ production, VCAM-1 expression, and tracheal hyperreactivity. In the present study, murine model of asthma, prepared and characterized as above, was designed to monitor the effect of Chinese tradition medicine of XQLT on allergen-induced lymphocyte proliferation and airway inflammation in dust mite (Der p)-sensitized BALB/c mice. We have shown that there was

significant decrease in numbers of total cells and percentage of eosinophil in the BAL of sensitized mice which were treated 1 g/kg of XQLT 30 min allergen challenge. On the contrary, the percentage of lymphocytes in the BAL were significantly increased in group A as compared to that of PBS group. Moreover, flow cytometry analysis has showed that this increase of lymphocyte percentage in BAL was mainly due to increase in the percentage of CD4+ T cells subset and, in minor part, to double-negative T cells. Therefore, further studies are needed to verify the possibility that XQLT may be able to revert Th2 dominant type of allergic inflammation to Th1 phenotype production.

T-lymphocytes, of a Th2-like phenotype [13], are thought to play an important role in orchestrating the asthmatic inflammatory response. When atopic asthmatic expose to allergen, it is found that CD4+ T-cells are depleted in the circulation and sequestrated in the lung [14]. Moreover, an elevation in double negative T lymphocytes was observed in subjects who develop isolated early asthmatic response but not in late-phase responder. It is proposed that CD4-/CD8- cells function to downregulate or suppress the late inflammatory response [15]. Therefore, the immunomodulatory role of XQLT in the allergen-sensitized mice, possible through regulation of T-lymphocyte activation.

It has been reported that mice given single dose of XQLT (8g/kg daily, p.o.) would induced a high energy state and not cause any harmful effect of the liver in mice. With the aim of evaluating whether there is any long term prophylactic effect of XQLT on allergen-induced airway inflammation, we had administrated 1 g/ml/kg of mycelial extract of XQLT to sensitized mice every other day for six times before AC. Though there was lower numbers of total cells in BAL of group C sensitized mice as compared to that of PBS group, the percentage of eosinophils and cellular distributions were not significantly changed during peak inflammatory state on day 3 after AC between these two groups. The different response of these two protocols, one dose of XQLT just after AC

versus six doses of XQLT before AC, in sensitized mice is, at the present moment, unable to explain.

There were only few reports on the immunomodulatory effect of XQLT, and its mechanism is still far from clear. Eda reported XQLT did not have significant suppressive effects on IL-2 production and IL-2 receptor expression from PBMC of intractable asthma stimulated by candida. But XQLT have suppressive effects on both LTC4 and O2- production. These result suggest that XQLT has inhibitory effect of LTC4 and O2- production by neutrophils prevent prolonged broncho-contriction and irresversible changes in small airways. [Eda] The effect of XQLT on human basophils growth in vitro using conditioned medium of T cell leukemia cell lineMo as a growth factor. It has found that XQLT, as well as other anti-allergic agent such as ketotifen, was able to inhibited the growth of human basophils growth and histamine release detected from culture supernatant. [Tanno et al] In another study to compare the different activity of various kind of Chinese herb medicine, as oppose to the other medicine tested in the study, XQLT has shown litter effect of increased synthesis of G-CSF of PBMC from normal volunteers. [Yamashiki et al] In another study, we have found that XQLT was able to inhibit LTB4 production, while had no effect of histamine release in peripheral blood mononuclear cells from Der p-sensitized asthmatic children (unpublished data). Therefore, the different responses between group A (treatment) and group B (prophylactic) may be due to the anti-inflammatory effect of XQLT on the airway inflammation and suppression of CD4+ T-cells, while XQLT may have no effect on the immediate type hypersensitivity reaction which is mediated by preformed granules released from mast cells and basophils during allergen challenge.

In summary, we found that mycelial extract of XQLT administrated to allergen-sensitized mice 12 h after allergen challenge was able to suppress airway inflammation by decreasing numbers of total cells and percentage of eosinophils in the BAL fluids, and downregulate the

allergen- or mitogen- induced intrapulmonary lymphocytes response. This immunomodulatory effect of XQLT may through regulation of T-lymphocytes by elevation or activation of CD4+ and doble negative T cell subsets. While long term administration of XQLT to sensitized mice before AC did not have the same inhibitory effect on airway inflammation as XQLT given after AC. The mechanism of these two different responses of XQLT is, at the present moment, unclear. Further studies are needed to know the precise role of this herb medicine in the treatment of asthma.

Acknowledgements

This work was partly supported by grant CCMP 87-RD-033, Committee of Chinese medicine and pharmacy, Department of Health, Taiwan, Republic of China.

(5) Reference

1. Eda R. Studies on the treatment for intractable asthma part2. Evaluation of the pharmacological action of various Kampo medicines on lymphocyte and neutrophil functions in intractable asthmatics. Okayama Igakkai Zasshi 1990,120:1323-1332
2. Nagai T, Yamada H. In vivo anti-influenza virus activity of Kempo (Japanese Herbal) medicine Sho-Seiryu-To and its mode of action. Int J Immunopharmac 1994;16:605-613.
3. Tanno Y, Shindo Y, Takishima T. Modulation of human basophil growth in vitro by Xiao-Qing-Long-Tang (Syo-seiry-to), Chai-Pu-Tang (Saiboku-to), Qing-Fei-Tang (Siehai-to), Baicalein and ketotifen. Am J Chinese Med 1989;17:45-50.
4. Yamashiki M, Asakawa M, Kayyaba Y, Kosaka Y, Nishimura A. Herbal medicine Sho-saiko-to induces in vitro granulocyte colony-

stimulating factot production on peripheral blood mononuclear cells. J Clin Lab Immunol 1992;37:83-90.

5. Minematsu S, Sudo K, Watanabe M, Taki M, Kanitani M, Fujii Y, Single oral dose toxicity study of Tsumura Sho-Seiryu-To TJ-19 in Rats. Pharmacometrics 1992;43:15-17)

6. Ikeda K, Wu DZ, Ishigaki M, Sunose H, Takasaka T. Inhibitory effects of Sho-seiryu-to on acetylcholine-induced responses in nasal gland acinar cells. Am J Chinese Med 1994;22:191-6 [XQLT attenuated the secretion of water and electrolytes from the nasal galnds through an anti-cholinergic effect.]

7. Banerjee P, Xu XJ, Poulter LW, Rustin MHA. Changes in CD23 expression of blood and skin in atopic eczema after Chinese herb therapy. Clin Exp Allergy 1998;28:306-314 [Chinese herb medicine treatment lead to a significant decrease in the number of CD23+ mature macrophages in the skin as well as a reduction in the level of expression of this moiety.]

8. Yamagiwa. M. Acoustic evaluation of the efficacy of medical therapy for allergic nasal obstruction . Eur Arch Oto-Rino-Larygo 1997;1:S82-84.

9. Tannao Y, Shindoh Y, Takishima T. Modulation of human basophil grouwth in vitro by xiao-qing long-tong (sho-seiryu-to) chai pu-tang (saiboko-to), qing-fei tang(seihai-to), baicalen and ketotifen. Am J Chinese Med 1989, 17:45-50. Histamin release and basophile number was inhibited by XQLT.

10. YU, S.C. LEE, J.Y. WANG, T.R. HSIUE, H.Y. LEI, J. Immunol. 156 1923-1930 (1996).

11. YU, B.C. YANG, S.C. LEE, J.Y.WANG, T.R. HSIUE, H.Y. LEI, Int. Arch. Allergy Immunol. 112 73-82 (1997).

12. 呂克蝻，謝貴雄，臺北市學童過敏病11年間之變化，中兒醫誌

29:104-109，1988。

13. 呂克蝻，謝貴雄，過去二十年臺灣過敏病罹患病率之增加，中兒醫誌 36:151，1995。

14. Harrison's Principles of Internal Medicine.13th Edition, CD-ROM Version 3.7, McGraw-Hill, Inc., 1995。

15. 鄧鐵濤，郭振球，中醫診斷學，知音出版社，台北，p.585，1989。

16. Keven JT, Gary KD, Geoffrey AS, Michael PA, Ann JW. Prevalence of asthma in the South Fore people of the Okapa District of papua New Guinea Int Arch Allergy Appl Immunol 77:158, 1985。

17. Skarpass IJK, Gulsvik A. Prevalence of bronchial asthma and respiratory symptoms in school children in Oslo. Allergy 40:295，1985。

18. Kaplan BA，Mascie-Taylor CGN. Biosocial factors in the epidemiology of childhood asthma in a British national sample. J. Epidermiol Comm Health 139:152，1985。

19. Speight ANP, Lee DA, Hey EN Underdiagnosis and undertreatment of asthma in childhood Br Med J 286:1253-1256，1983。

20. 王秀敏，小兒氣喘，臨床醫學 20:47-52，1987。

21. KF CHUNG. Role of inflammation in the hyperreactivity of the airways in asthma.Thorax 41:657-662, 1986。

22. J. Pepys, B. J. Hutchcroft. Bronchial provocation test in etiologic diagnosis and analysis of asthma. AM REV RISPIR DIS 112:829-859, 1975。

23. Penny A. Hutson, Stephen T. Holgate, Martin K. Church. The effect of cromolyn sodium and albuterol on early and late phase bronchoconstriction and airway leukocyte infiltration after allergen challenge of nonanesthetized guinea pigs. AM REV RESPIR DIS

138:1157-1163, 1988 。

24. H. Booij-Noord, N. G. M. Orie, K. de Vries. Immediate and late bronchial obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. J. Allergy Clin. Immunol 48(6):344-354, 1971 。

25. Peter SP ， Schleimer RP, Naclerio RM. The pathophysiology of human mast cells: in vitro and in vivo function. AM REV RESPIR DIS 135:1196-1200, 1987 。

26. Tonnel AB, Joseph M, Gosset PH, Fournier E, Capron A. Stimulation of alveolar macrophages in asthmatic patients after local provocation test. Lancet 2:1406-1408 ， 1983 。

27. AB Kay, CJ Corrigan. Eosinophils and neutrophils. British Medical Bulletin 48(1):51-64 ， 1992 。

28. Jan G. R. De Monchy, Henk F. Kauffman, Per Vengek, Gerard H. Koeter, Henk M. Jansen, Henk J. Sluiter, Klaas De Vries. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. AM REV RESPIR DIS 131:373-376, 1985 。

29. CJ Corrigan, AB Kay. Role of T-lymphocytes and lymphokines. British Medical Bulletin 48(1):72-84, 1992 。

30. K F Chung, P J Barnes. Role of inflammatory mediators in asthma. British Medical Bulletin 48(1):135-148, 1992。

31. 馬建中，何東燦，邱年永，中醫內科學，正中書局，台北，pp.119-120，1986。

32. 張伯臾，董建華，周仲瑛，中醫內科學，知音出版社，台北，pp.95-121，1989。

33. 明，虞摶，醫學正傳，新文豐出版公司，台北，pp.260-261，1981。

34. 清，葉天士，臨證指南醫案，新文豐出版公司，台北，

pp.299,302，1980。

35. 唐，王冰，黃帝內經素問，旋風出版社，台北，pp.46,155,191-193,199,238，1979。

36. 明，馬元台、張隱庵合著，黃帝內經素問靈樞合編，台聯國風出版社，台北，pp.60,179,258-259,296，1981。

37. 漢，張仲景，傷寒論，台聯國風出版社，台北，pp.113,126-127,127,130,132,138,196,215，1974。

38. 漢，張仲景，仲景全書，集文書局，台北，pp.358,375-376，1983。

39. 李永清，《傷寒論》咳喘證治，福建中醫藥，19(2):20-21，1988。

40. 隋，巢元方，諸病源候論，文光圖書有限公司，台北，pp.188,201，1981。

41. 唐，王燾，外臺秘要，國立中國醫藥研究所，台北，pp.266,291-293，1985。

42. 清，陳夢雷，圖書集成醫部全錄，新文豐出版公司，台北，1979。

43. 宋，曹孝忠等，聖濟總錄，新文豐出版社，台北，p.433，1978。

44. 元，朱丹溪，丹溪心法，五洲出版社，台北，pp.339-340，1969。

45. 元，朱丹溪，脈因證治，五洲出版社，台北，pp.85-86。

46. 明，秦景明，症因脈治，旋風出版社，台北，pp.117,120,127，1979。

47. 明，戴元禮，秘本證治要訣及類方，文光圖書有限公司，台北，p.64，1981。

48. 明，張景岳，景岳全書，台聯國風出版社，台北，pp.345,348，1980。

49. 明，李中梓，醫宗必讀，文光圖書有限公司，台北，p.44，1971。

50. 明，孫東宿，赤水玄珠，台聯國風出版社，台北，1978。

51. 清，李用粹，證治彙補，文光圖書有限公司，台北，pp.278-279，1984。

52. 清，沈金鰲，沈氏尊生書，自由出版社，台北，pp.46-47，1988。

53. 清，林佩琴，類證治裁，新文豐出版社，台北，p.113，1980。

54. 張豐強，中國名方應用大全-現代方證學，中國醫藥科技出版社，北京，pp.31-37，1992。

55. 遼寧省中醫研究院編，傷寒論方證研究，遼寧科學技術出版社，瀋陽，pp.35-42，1984。

56. 陶凱，于文生，小青龍湯夏用治療哮喘39例，山東中醫學院學報 16(1):43，1992。

57. 張建國，許得勝，小青龍口服液治療支氣管哮喘的療效觀察，上海中醫藥雜誌 2:25-26，1993。

58. 黃先善，小青龍湯在慢性支氣管炎急性發作期的臨床應用，浙江中醫學院學報 17(5):12-13，1993。

59. 李學清，小青龍湯治療小兒喘息性肺炎11例，中西醫結合雜誌 5(5):276，1985。

60. 張玉珍，小青龍湯為主治療小兒支氣管肺炎102例，遼寧中醫雜誌 20(1):33-34，1993。

61. 田道正，小青龍湯治療過敏性鼻炎的經驗，山東中醫雜誌 12(4):23-24，1993。

62. 賀哲，李志遠，王康社，加減小青龍湯治療咳喘181例，陝西中醫 13(7):314，1992。

63. 丁建華，小青龍湯加減治療咳喘258例，山東中醫雜誌 13(8):342，1994。

64. 舒應德，小青龍湯治療久咳，四川中醫 8(1):26，1990。

65. 黃泰康，常用中藥成分與藥理手冊，中國醫藥科技出版社，北京，pp.178-194,526-547,745-750,779-783,1304-1309,1624-1630，1994。

66. 孫靜芸，麻黃新的有效成分研究，中草藥 14(8):9-10,14，1983。

67. 林景彬，常用中藥藥理與運用，中國醫藥學院，台中，pp.3-13,13-17,55-61,429-449，1985。

68. 林啟壽，庄麗，王慕鄒，李崢，白芍和赤芍成分的比較，藥學學報 4(1):17-24，1956。

69. 陳榮福，中藥藥理學，國立中國醫藥研究所，台北，pp.107-110,132-139,228-232,299-303，1991。

70. 中藥臨床應用，啟業書局，台北，pp.160-162,334-336，1987。

71. 許鴻源，中藥成分最近的研究，國立中國醫藥研究所，台北，pp.32-33,46-48，1968。

72. 後藤實，桂皮和桂枝，醫學文摘(Ⅲ) 8(3):99，1965。

73. 袁阿興，覃凌，韋善新，康書華，姜達衢，中藥桂枝化學成分的研究，中藥通報 9(3):31-32，1984。

74. 王浴生，中藥藥理與應用，人民衛生出版社，北京，pp.862-867，1983。

75. 王嶽，黃爾秀，王瑜欽，林益明，吳肯堂，70種藥用植物抗菌效能的試驗，植物學報 3(2):121-131，1954。

76. 陳奇，中國名方藥理與應用，南天書局，台北，pp.44-45，1993。

77. 王筠默，顧月芳，張海桂，小青龍湯及其主要組成藥的平喘作用，藥學通報 17(5):51-52，1982。

78. 施賽珠，沈自尹，某些平喘方藥的臨床應用和研究的進展，中醫雜誌 4:74-75，1985。

79. 丁培植，日本對小青龍湯治療小兒支氣管哮喘研究概況，中西醫結合雜誌 7(1):60-61，1987。

80. W. M. Abraham, J. C. Delehunt, L. Yerger, B. Marchette. Characterization of a late phase pulmonary response after antigen challenge in allergic sheep. AM REV RESPIR DIS 128:839-844, 1983。

81. 70.Mark P. Shamoain, B. Lyn Behrens, Gary L. Larsen, Peter M. Henson. An animal model of pulmonary responses to alternaria challenge. AM REV RESPIR DIS 126:493-498, 1982。

82. F. Chung, A. B. Becker, S. C. Lazarus, O. L. Frick, J. A. Nadel, W. M. Gold. Antigen-induced airway hyperressponsiveness and pulmonary inflammation in allergic dogs. J. Appl. Physiol. 58(4):1347-1353, 1985。

83. Hamel, C. S. McFarlane, A. W. Ford-Hutchinson. Late pulmonary responses induced by ascaris allergen in conscious squirrel monkeys. J. Appl. Physiol. 61(6):2081-2087, 1986。

84. Kallos, L. Kallos. Experimental asthma in guinea pigs revisited. Int Archs Allergy Appl Immunol 73:77-85, 1984。

85. 74.陳奇，中藥藥理研究方法學，人民衛生出版社，北京，pp.85-87，1993。

86. 75.田_嘉雄，實驗動物學各論，朝倉書局，東京，pp.66-76，1972。

87. 76.Hideya Iijima, Munehiko Ishii, Kohei Yamauchi, C-L Chao, Keiji Kimura, Sanae Shimura, Yuriko Shindoh, Hiroshi Inoue, Suetsugu Mue, Tamotsu Takishima. Bronchoalveolar lavage and histologic characterization of late asthmatic response in guinea pigs. AM REV RESPIR DIS 136:922-929, 1987。

88. 77.Akira Ishii, Koji Ito, Yoshitaka Ino, Terumasa Miyamoto. Experimental asthma in guinea pigs sensitized with mites. Int Arch

Allergy Appl Immunol 89:400-403, 1989 。

89. F. Walls, Y. K. Rhee, D. J. Gould, C. Walters, C. Robinson, M. K. Church, S. T. Holgate. Inflammatory mediators and cellular infiltration of the lungs in a guinea pig model of the late asthmatic reaction. Lung 169:227-240, 1991 。

90. Penny A. Hutson, Martin K. Church, Timothy P. Clay, Peter Miller, Stephen T. Holgate. Early and late-phase bronchoconstriction after allergen challenge of nonanesthetized guinea pigs. AM REV RESPIR DIS. 137:548-557, 1988 。

91. Haruhito Sugiyama, Ryosuke Eda, Chiharu Okada, Russell J. Hopp, Againdra K. Bewtra, Robert G. Townley. Eosinophil accumulation and activation in antigen-induced late asthmatic response in guinea pigs. Journal of Asthma. 32(1):37-45, 1995 。

92. Chand N, Harrison JE, Rooney SM, Nolan KW, De Vine CL, Jakubicki RG, Pillar J, Diamantis W, Sofia RD. Allergic bronchial eosinophilia: a therapeutic approach for the selection of potential bronchial anti-inflammatory drugs. Allergy 48:624-626, 1993 。

93. 谿忠人，醫療用漢方製劑療法，現代醫療 17(6)： 1121 ， 1985 。

Appendix

The Bioactivity Components of XQLT:

1. Herba Ephedrae: *1-ephedrine, d-pseudoephedrine, 2,3,4,5,6-tetramethyl-pyrazine*
2. Radix Paeoniae: *paeoniflorin, paeonol, benzoic acid, b-sitosterol*
3. Herba Asari: *methyleugenol, safrole, pinene, eucarvone, 1-asarinin.*
4. Rhizoma Zingiberis: *zingiberone, b-bisabolene, a-curcumene, b-sesquiphellandrene, zingiberol*
5. Radix Glycyrrhizae: *triterpene saponin, glycyrrhetic acid, flavones,*

licoricone

6. Ramulus Cinnamomi*: cinnamic aldehyde, trans-cinnamic acid, coumarin, b-sitos-terol protocatechuic acid*
7. Fructus Schisandrae: *schizandrin, deoxyschizandrin, gemisin, chamigrenal, chamigrene, sesquicarene.*
8. Rhizoma Pinelliae*: b-sitosterol, b-sitosterol D-glucoside*

Table 1. Change in total numbers and cellular distribution in BAL of sensitized mice after Der-p challenge.

Hours postchallenge	BAL cells (x 10^5/ml)	Cell Nos (x 10^4/ ml) macrophages	lymphocytes	neutrophils	eosinophils
saline challenge	3.1 ± 0.5	29.5 ± 0.0 (95.0 ± 0.0)	0.9 ± 0.0 (3.0 ± 0.0)	0.6 ± 0.0 (2.0 ± 0.0)	0
Der-p challenge					
1	4.0 ± 1.0	37.6 ± 0.4 (90.0±1.0)	1.4 ± 0.2 (3.5 ± 0.5)	1.0 ± 0.6 (2.5 ± 1.5)	0
6	20.8 ± 11.6	77.0 ± 8.3 (37.0 ± 4.0)	30.2 ± 1.0 (14.5 ± 1.0)	100.9 ± 7.3 (48.5 ± 3.5)	0
24	11.1 ± 1.8	26.8 ± 7.4 (22.9 ± 2.5)	14.3 ± 4.1 (12.3 ± 1.3)	50.8 ± 6.0 (57:2 ± 2.4)	12.1 ± 3.0 (7.7 ± 3.1)
48	8.5 ± 1.6	10.7 ± 4.1 (12.1 ± 2.6)	28.6 ± 7.46.8 (33.3 ± 1.8)	36.9 ± 3.8 (44.2 ± 3.9)	8.9 ± 1.3 (10.5 ± 0.5)
72	9.3 ± 3.3	40.0 ± 6.5 (43.0 ± 7.0)	10.2 ± 0.9 (11.0 ± 1.0)	37.7 ± 7.9 (40.5 ± 8.5)	5.1 ± 0.5 (5.5 ± 0.5)

Table 2.

A

Drug	BAL cells (x 10^5 / ml)	Cell Nos (x 10^4 / ml)			
		macrophages	lymphocytes	neutrophils	eosinophils
Naive	2.5 ± 0.3	23.7 ± 0.7 (94.7 ± 3.2)	1.2 ± 0.4 (4.7 ± 1.7)	0.2 ± 0.2 (0.7 ± 1.0)	0
Naïve + XQLT 24 h.	6.1 ± 0.8	48.0± 4.5 (78.7 ± 0.3)	12.6 ± 4.2 (20.4 ± 7.0)	0.4 ± 0.3 (0.7 ± 0.5)	0

B

Drug	BAL cells (x 10^5 / ml)	Cell Nos (x 10^4 / ml)			
		macrophages	lymphocytes	neutrophils	eosinophils
Naive	3.47 ± 0.2	30.4 ± 1.0 (87.7 ± 2.9)	3.9 ± 1.6 (11.3 ± 1.7)	0.3 ± 0.5 (1.0 ± 1.4)	0
Naïve + XQLT 24 h.	7.1 ± 0.3	41.2± 6.4 (58.0 ± 9.0)	28.0 ± 8.2 (39.5 ± 11.5)	1.8 ± 1.8 (2.5 ± 2.5)	0

Table 3.

A

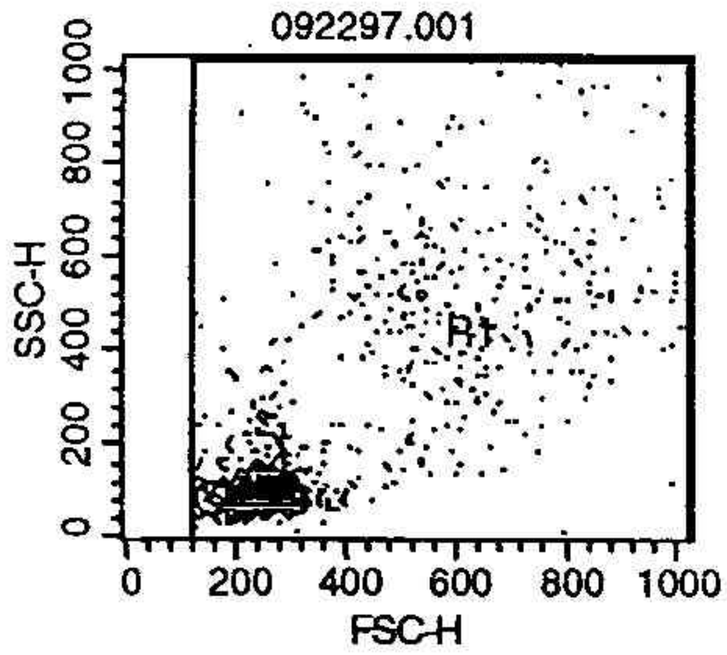
Hours postchallenge	BAL cells (x 10^5/ml)	Cell Nos (x 10^4/ ml)			
		macrophages	lymphocytes	neutrophils	eosinophils
Saline challenge	4.0 ± 0.4	36.8 ± 3.3 (92.0 ± 1.0)	2.8 ± 0.7 (7.0 ± 1.0)	0.4 ± 0.0 (1.0 ± 0.0)	0
給予小青龍湯 (1g/kg) , 24 h.	2.5 ± 0.3	4.7 ± 0.2 (19.0 ± 3.0)	20.2 ± 3.3 (80.5 ± 3.5)	0	0
給予小青龍湯 (1g/kg) , 48 h.	4.2 ± 1.4	3.5 ± 1.0 (8.5 ± 1.0)	38.0 ± 13.0 (90.0 ± 1.0)	0.6 ± 0.0 (1.5 ± 0.5)	0

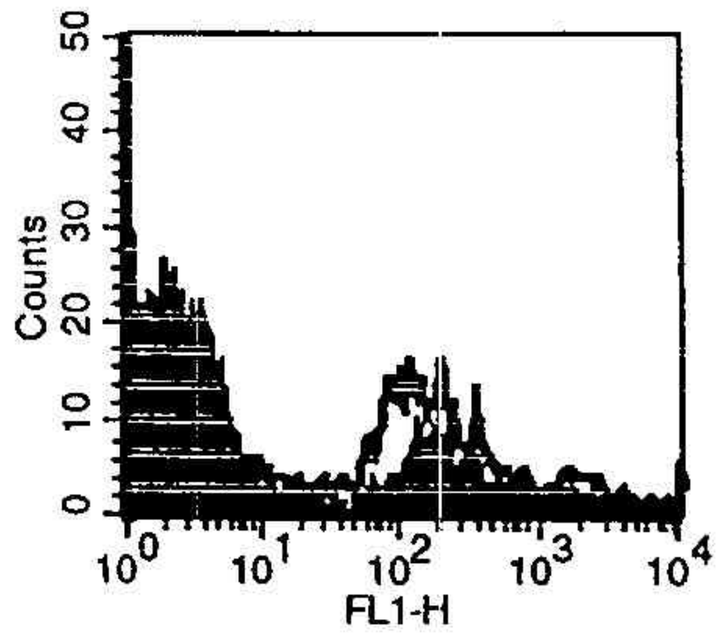
B

Drug	BAL cells (x 10^5 / ml)	Cell Nos (x 10^4 / ml)			
		macrophages	lymphocytes	neutrophils	eosinophils
Saline challenge	5.4 ± 0.6	48.3 ± 0.3 (89.5 ± 0.5)	4.3 ± 0.5 (8.0 ± 1.0)	1.1 ± 1.0 (2.0 ± 0.0)	0
給予小青龍湯 (0.2g/kg) 5 天, saline; 24 h.	5.2 ± 0.0	10.9 ± 0.0 (21.0 ± 0.0)	40.6 ± 0.0 (78.0 ± 0.0)	0	0
給予小青龍湯 (1g/kg) 5 天 saline ; 48 h.	4.2 ± 0.2	7.1 ± 1.3 (17.0 ± 3.0)	33.0 ± 1.1 (78.5 ± 2.5)	1.9 ± 0.2 (4.5 ± 0.5)	0
給予小青龍湯 (1g/kg) 5 天 saline ; 72 h.	12.4± 0.0	16.1 ± 0.0 (13.0 ± 0.0)	104.0 ± 0.0 (84.0 ± 0.0)	3.7 ± 0.0 (3.0 ± 0.0)	0

Fig. 1

Naive





CD3 Tcells

Naive + XQLT 24h

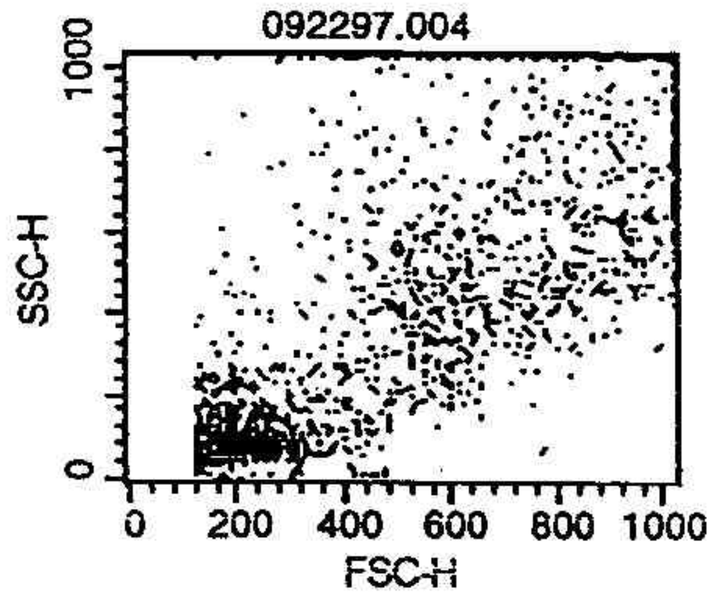


Table 4.

Drug	BAL cells (x 10^5 / ml)	Cell Nos (x 10^4 / ml)			
		macrophages	lymphocytes	neutrophils	eosinophils
Saline challenge	2.9 ± 0.8	26.4 ± 2.3 (91.0 ± 0.8)	1.7 ± 0.2 (6.0 ± 0.8)	0.9 ± 0.2 (3.0 ± 0.8)	0
Der-P; 24 h.	9.6 ± 0.0	24.0 ± 3.8 (25.0 ± 4.0)	10.1 ± 2.4 (10.5 ± 2.5)	51.8 ± 1.9 (54.0 ± 2.0)	10.1 ± 0.5 (10.0 ± 3.0)
30 min.前給予小青龍湯(1g/kg)後 Der-P; 24 h.	6.9 ± 1.0	5.5 ± 0.8 (8.0 ± 0.0)	25.4 ± 3.6 (37.0 ± 5.1)	36.7 ± 8.5 (52.5 ± 5.4)	1.9 ± 0.6 (3.0 ± 1.4)
給予小青龍湯 (1g/kg) 5 天 Der-P ; 24 h.	14.8 ± 0.4	42.2 ± 0.7 (28.5 ± 0.5)	11.2 ± 5.2 (7.5 ± 3.5)	72.5 ± 8.9 (49.0 ± 6.0)	22.2 ± 4.4 (15.0 ± 3.0)
給予小青龍湯 (1g/kg) 5 天 Der-P ; 72 h.	5.6± 0.0	12.6 ± 1.4 (22.5 ± 2.5)	37.0 ± 2.2 (66.0 ± 4.0)	3.4± 0.6 (6.0 ± 1.0)	3.1 ± 0.3 (5.5 ± 0.5)
Der-P; 24 h.後給予小青龍湯 (1g/kg) 48 h.	11.5 ± 1.7	41.1 ± 8.5 (33.7 ± 7.4)	53.7 ± 7.2 (46.7 ± 6.2)	8.1 ± 3.3 (7.0 ± 2.8)	16.1 ± 2.8 (14.0 ± 2.4)

Table 5.

Drug	BAL cells (x 10^5 / ml)	Cell Nos (x 10^4 / ml)			
		macrophages	lymphocytes	neutrophils	eosinophils
Saline challenge	4.0 ± 0.4	36.8 ± 3.3 (92.0 ± 1.0)	2.8 ± 0.7 (7.0 ± 1.0)	0.4 ± 0.0 (1.0 ± 0.0)	0
Der-P; 24 h.	10.1 ± 2.2	19.3 ± 3.5 (19.3 ± 1.7)	10.9 ± 2.8 (12.7 ± 1.7)	58.0 ± 0.8 (50.4 ± 13.5)	8.8 ± 4.1 (9.7 ± 2.6)
30 min. 前給予小青龍湯(1g/kg)後, Der-p; 24 h.	6.3 ± 2.4	6.2 ± 0.4 (8.5 ± 0.5)	25.9 ± 12.5 (34.0 ± 6.0)	37.0 ± 8.2 (53.5 ± 6.5)	2.9 ± 1.0 (4.0 ± 0.0)
給予小青龍湯 (1g/kg) , 24 h.	2.5 ± 0.3	4.7 ± 0.2 (19.0 ± 3.0)	20.2 ± 3.3 (80.5 ± 3.5)	0	0
Der-P; 48 h.	6.9 ± 0.1	6.6 ± 1.8 (9.5 ± 2.5)	21.8 ± 1.4 (31.5 ± 1.5)	33.1 ± 4.2 (48.0 ± 5.0)	7.6 ± 0.8 (11.0 ± 0.5)
Der-P 24h. 後給予小青龍湯 (1g/kg) ; 24 h.	11.0 ± 2.3	11.9 ± 3.6 (10.7 ± 0.9)	51.1 ± 11.2 (46.3 ± 0.5)	18.3 ± 4.1 (18.0 ± 6.7)	28.7 ± 12.1 (25.0 ± 5.4)
30 min. 前給予小青龍湯(1g/kg)後, Der-p; 48 h.	6.5 ± 1.5	4.2 ± 0.7 (6.5 ± 0.5)	45.4 ± 11.4 (69.5 ± 1.5)	13.1 ± 2.1 (20.5 ± 1.5)	2.8 ± 1.3 (4.0 ± 1.0)
給予小青龍湯 (1g/kg) , 48 h.	4.2 ± 1.4	3.5 ± 1.0 (8.5 ± 1.0)	38.0 ± 13.0 (90.0 ± 1.0)	0.6 ± 0.0 (1.5 ± 0.5)	0

Table 6.

Drug	BAL cells (x 10^5 / ml)	Cell Nos (x 10^4 / ml)			
		macrophages	lymphocytes	neutrophils	eosinophils
Saline challenge	5.4 ± 0.6	48.3 ± 0.3 (89.5 ± 0.5)	4.3 ± 0.5 (8.0 ± 1.0)	1.1 ± 1.0 (2.0 ± 0.0)	0
Der-P; 24 h.	13.7 ± 2.5	37.0 ± 2.7 (27.0 ± 2.0)	19.9 ± 2.1 (14.5 ± 1.5)	63.7 ± 4.8 (46.5 ± 3.5)	16.4 ± 4.1 (12.0 ± 3.0)
給予小青龍湯 (0.2g/kg) 5 天, Der-p; 24 h.	8.6 ± 0.2	12.0 ± 1.7 (14.0 ± 2.0)	11.2 ± 2.6 (13.0 ± 3.0)	57.6 ± 3.4 (67.0 ± 4.0)	5.6 ± 0.4 (6.5 ± 0.5)
給予小青龍湯 (0.2g/kg) 5 天, Der-p; 48 h.	12.9 ± 4.0	31.6 ± 12.3 (24.5 ± 9.5)	50.3 ± 7.7 (39.0 ± 8.5)	41.3 ± 20.3 (32.0 ± 16.0)	7.1 ± 0.6 (5.5 ± 0.5)
給予小青龍湯 (0.2g/kg) 5 天, saline; 24 h.	5.2 ± 0.0	10.9 ± 0.0 (21.0 ± 0.0)	40.6 ± 0.0 (78.0 ± 0.0)	0	0
Der-P; 24 h.後 給予小青龍湯 (1g/kg) 24 h.	17.1 ± 1.0	24.8 ± 0.9 (14.5 ± 0.5)	64.1 ± 2.6 (37.5 ± 2.1)	50.4 ± 2.6 (29.5 ± 1.5)	31.6 ± 0.9 (18.5 ± 0.5)
Der-P; 24 h.後 給予小青龍湯 (1g/kg) 48 h.	11.2 ± 0.0	19.0 ± 3.4 (17.0 ± 3.0)	56.0 ± 7.8 (50.0 ± 7.0)	22.4 ± 11.2 (20.0 ± 10.0)	14.6 ± 0.0 (13.0 ± 0.0)
給予小青龍湯 (1g/kg) 5 天 saline ; 48 h.	4.2 ± 0.2	7.1 ± 1.3 (17.0 ± 3.0)	33.0 ± 1.1 (78.5 ± 2.5)	1.9 ± 0.2 (4.5 ± 0.5)	0
給予小青龍湯 (1g/kg) 5 天 saline ; 72 h.	12.4± 0.0	16.1 ± 0.0 (13.0 ± 0.0)	104.0 ± 0.0 (84.0 ± 0.0)	3.7 ± 0.0 (3.0 ± 0.0)	0

	Total cells/ml $\times 10^5$	Macrophage $\times 10^5$	Lymphocte $\times 10^5$	Neutrophil $\times 10^5$	Eosinophil $\times 10^5$
長期 PBS , sensi.2*, it 48hs	19.88 ± 8.50	4.82 ± 2.28	3.68 ± 1.39	1.63 ± 1.56	9.66 ± 6.49
長期小青龍湯,sensi.2*,it 48hs	7.45 ± 2.39	2.72 ± 0.81	1.88 ± 1.16	0.64 ± 0.22	2.19 ± 0.62
長期小青龍湯,sensi.2*,it 48hs	5.48 ± 2.79	2.83 ± 0.89	2.71 ± 1.36	1.21 ± 0.58	2.03 ± 1.25

	Total cells/ml $\times 10^5$	Macrophage $\times 10^5$	Lymphocte $\times 10^5$	Neutrophil $\times 10^5$	Eosinophil $\times 10^5$
長期 PBS, sensi.2*,it 48hs	15.7 ± 3.5	5.17 ± 0.24	3.09 ± 0.76	4.91 ± 2.57	2.50 ± 0.259
長期小青龍湯,sensi.2*,it 48hs	15.1 ± 1.8	2.93 ± 0.73	1.98 ± 0.75	6 .07 ± 2.03	3.72 ± 0.96
長期 PBS,sensi.2*,it 4ds	9.6 ± 2.9	3.33 ± 1.19	2.45 ± 0.64	0.44 ± 0.35	3.55 ± 1.27
長期小青龍湯,sensi.2*,it 4ds	12.8 ± 5.7	3.55 ± 1.27	3.00 ± 2.30	0.33 ± 0.25	5.27 ± 2.64
長期 PBS,sensi.2*,it 8ds	6.9 ± 3.7	3.49 ± 2.32	2.2 ± 1.00	0.19 ± 0.30	0.99 ± 0.073
長期小青龍湯,sensi.2*,it 8ds	5.5 ± 2.4	2.08 ± 1.22	1.80 ± 1.10	0.07 ± 0.06	1.59 ± 0.71

FACScan

		Events	1 %	2. %	3. %	4. %
長期 PBS	Control	1837	0.54	0.76	97.22	1.47
	TCR	1776	0	0.56	36.37	63.06
	CD_8	1665	0	0.66	82.82	16.52
	IgG	1623	0	0.86	38.88	60.26
	TCR+CD_4	1676	0.60	36.10	40.57	22.73
長期小青龍湯	Control	2249	0.09	0.67	98.18	1.07
	TCR	1952	0	0.87	34.68	64.45
	CD_8	2071	0	0.53	85.08	14.39
	IgG	2113	0	0.43	58.26	41.32
	TCR+CD_4	1980	0.56	31.57	40.96	26.92
長期小青龍湯	Control	3118	0.61	0.61	98.11	0.66
	TCR	2118	0.14	0.33	20.68	78.85
	CD_8	2121	0.42	0.42	78.03	21.12
	IgG	2031	0.10	0.25	63.42	36.24
	TCR+CD_4	2078	0.43	54.86	20.70	24.01

		Events	1.(PE) %	2. %	3. %	4.(FITC) %
PBS 48hs	Control	2105	0.71	1.81	95.34	2.14
	CD_3+ CD_4 (FITC) (PE)	1205	2.07	32.20	37.34	28.38
	CD_3+CD_8 (PE) (FITC)	2315	45.75	22.07	30.06	2.12
小青龍湯 48hs	Control	19287	0.62	0.99	97.51	0.88
	CD_3+ CD_4 (FITC) (PE)	1620	0.68	31.48	37.10	30.74
	CD_3+CD_8 (PE) (FITC)	1892	34.83	18.32	44.23	2.62

Treat	*Mark*	*Gated events*	*Total %*	*Events*	*Gated %*	
隔天長期	Control	1837	0.27	27	1.47	
餵食 PBS	TCR	1776	11.2	1120	63.06	
10*	CD8	1665	2.75	275	16.52	
	IgG	1623	9.78	978	60.26	
	TCR+CD4	1676	3.81	381	22.73	
隔天長期	Control	2249	0.24	24	1.07	
小青龍湯	TCR	1952	12.58	1258	64.45	
10*	CD8	2113	2.98	298	14.39	
	IgG	2113	8.73	873	41.32	
	TCR+CD4	1980	5.33	533	26.92	
隔天長期	Control	2118	0.14	14	0.66	
小青龍湯	TCR	2118	16.7	1670	78.85	
10*	CD8	2121	4.48	448	21.12	
	IgG	2031	7.36	736	36.24	
	TCR+CD4	2087	5.01	501	24.01	
Sensi.2*	Control	2338	0.26	26	1.11	
it 前 1h PBS	CD8	3008	4.55	455	15.13	
	IgG	2561	15.01	1501	58.61	
Sensi. 2*	Control	2292	0.14	14	0.61	
it 前 1h 小青	CD8	2425	5.17	517	21.32	
	IgG	3024	15.61	1561	51.62	
Sensi. 2*	Control	3145	0.15	5	0.48	
it 後 24h	CD8	2142	4.82	482	22.5	
小青	IgG	2043	11.74	1174	57.48	
Sensi. 2*	Control	1814	0.12	12	0.66	
it 前 1h及	CD8	2454	6.1	610	24.86	
後 24h 小青	IgG	2601	13.13	1313	50.48	

	TCR	CD_3	CD_4 %	CD_8	IgG	備註
sensi.2* it 前 salin 48hrs				9.71	28.72	511
sensi.2* it 前小青龍湯 48hrs				8.47	33.48	512
sensi.2* it 後 24hs 小青龍湯 48hrs				9.47	35.07	513
sensi.2* it 前及後小青龍湯 48hrs				8.16	32.16	514

	TCR	CD_3	CD_4 %	CD_8	IgG	備註
naïve it 前 PBS		22.39	4.69	9.2	0.46	m5
naïve it 前小青龍湯		17.36	5.89	5.26	1.12	m6
sensi. it 前 PBS		47.38	27.59	10.4	2.65	m7
sensi. it 前小青龍湯		52.43	31.77	11.31	2.81	m8
naïve it 後 PBS		17.17	4.55	4.02	1.13	m9
naïve it 後小青龍湯		19.62	8.66	6.14	1.41	m10
senei. It 後 PBS		31.88	27.03	8.2	3.30	m11
sensi. it 後小青龍湯		49.41	24.91	13.21	1.81	m12

	TCR	CD_3	CD_4 %	CD_8	IgG	備註
長期 salin sensi.2* it 48hrs	35.94		25.98	6.52	15.82	505
長期小青龍湯 sensi. 2* it 48hrs	37.84		24.05	6.65	14.48	506
長期小青龍湯 snsi. 2* it 48hrs	39.87		24.71	5.87	11.26	507

	TCR	CD_3	CD_4 %	CD_8	IgG	備註
長期 salin sensi.2* it 48hrs		34.55	16.82	13.22		539
長期小青龍湯 sensi. 2* it 48hrs		43.66	21.28	13.81		540
長期 salin ,sensi.2* it 96hrs		46.76	34.28	10.77		541
長期小青龍湯 sensi. 2* it 96hrs		55.57	42.77	10.96		542