The Effect of *Sopungchungyoung-tang* on Activity of CD4 T cell

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Sopungchungyoung-tang (SCT) has been widely used in Korea as a treatment of atopic dermatitis. SCT consists of *Talcum, Rehmannia glutinosa, Angelica sinensis, Paeonia lactiflora, Cnidium officinale, Ledebouriella divaricata, Schizonepeta tenuifolia, Scutellaria baicalensis, Glycyrrhiza uralensis, Mentha arvensis, Cordyceps cicadae.* We examined the immunological effect of SCT in vitro. We studied about the effect of SCT on Th cells' differentiation. In the case of CD4 T cells under neutral condition where there was only rIL-2 stimulus, SCT inhibited IFN-γ secretion by 70-80 %. Likewise, SCT also inhibited the IL-4 secretion of neutral Th cells by 85-90 %. We also experimented with the polarized Th1 cells/ Th2 cells and their production of IFN-γ and IL-4, respectively. There also were inhibitory effects on the polarized cells like there was on neutral cells, they were not as strong on the polarized cells. Under Th1 polarized condition, SCT acted dose-dependently, while in Th2 cells, the IL-4 production was inversely proportional to the doses of SCT. From the current study, it can be concluded that SCT exerts inhibitory effects on cytokine production without interfering with immune cells' activity. The result that SCT inhibits IFN-γ and IL-4 confirms that it does have a effect on immunomodulation.

Key words : *Sopungchungyoung-tang*(SCT), CD4 T cell, INF-γ, IL-4

Introduction

Allergic rhinitis, asthma, and atopic dermatitis are among the most common causes of a chronic illnesses. And with these diseases increasing in prevalence, they inevidently take up a considerable amount of health care costs, imposing a heavy burden. In the United States, for example, the annual cost of asthma treatment alone is about $6 billion (Smith DH, 1997). A considerable body of data has accumulated for the past 15 years. Enough to show that atopic disorders are driven by the T helper 2 (Th2) subset of cluster of differentiation 4 (CD4) T cells. Mosmann et al. performed an experiment in mice and was the first to distinguish the two types of T helper clones, Th1 and Th2 cells, based on their profile of cytokine secretion (Mosmann T, 1986).

According to the researches on such allergies, it has been proposed that an imbalance between the Th1 / Th2 immune response profile is the immunologic basis of them. For instance, allergic diseases like asthma are thought to arise through type 2 responses. While on the other hand, many autoimmune diseases involve type 1 responses (O'Garra A, 1997).

When an immune reaction begun, which direction the immune reaction will take, in other words, which of the two Th cells (Th1 / Th2) will the precursor cell differentiate into, depends on the cytokine environment present during the priming of the precursor cells. The presence of IL-4 will promote differentiation into Th2 cells, whereas IL-12 and IFN-γ will drive precursor Th cells to differentiate into Th1 cells (Swain SL. 1995).

Th1 cells appeared to be the main effecters of the phagocyte-mediated host defense, through their production of Interferon-γ (IFN-γ) (Mosmann T, 1987; Yamamura M, 1991). Th2 cells were shown to produce Interleukin-4 (IL-4), IL-5, IL-13, IL-9 and IL-10. IL-4 and IL-13 induce the synthesis of Immunogloblin E (IgE) and IgG1 in mice, and the synthesis of IgE and IgG4 in humans; IL-5 enhances eosinophil differentiation and is a major eosinophil-activating cytokine.

It is a golden saying in oriental medicine, that "When the vital energy (Qi) is substantial and strong, no pathogenic factor shall break into your body". It is mentioned in "The Methods of Acupuncture", Huang Ti Nei Ching Su Wen (Ilza V, 1972), meaning that allergies only occur when the vital energy becomes exhausted, consequently weakening the immune system.

In this study, we examined the immunological effect of a traditional Korean herbal medicine, Sopungchungyoung-tang

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(SCT) in vitro. SCT has been widely used in Korea as a treatment of atopic dermatitis. SCT is a treatment for atopic dermatitis, which is caused by wind as pathogenic factor consumption on account of blood deficiency.

Atopic dermatitis, commonly referred to as eczema, is a chronic skin disorder categorized by scaly and itching rashes. According to oriental medicine, the cause of atopic dermatitis is the excess of fire leading to insufficiency of water, which is connected with blood deficiency. Wind syndrome can occur in case of extreme heat. As dry skin often makes the condition worse, SCT is very useful medicine for that condition. SCT is also an efficacious remedy for nourishing the blood to disperse wind.

Most of the SCT's materials have been known as working on immune system. There has already been studies in progress about the effects each separate herbs in SCT has on the immune system. (*Rehmannia glutinosa* (Kim H, 1998), *Angelica sinensis* (Wilasrusmee C, 2002; Weng XC, 1987), *Paeonia lactiflora* (Tomoda M, 1994; Tomoda M, 1993), *Cnidium officinale* (Lee GI, 1995; Tomoda M, 1992), *Schizonepeta tenuifolia* (Shin TY, 1999), *Glycyrrhiza uralensis* (Shimizu N, 1992; Yang G, 1990), *Mentha arvensis* (Shin TY, 2003), *Cordyceps cicadae* (Kuo YC, 2003; Weng SC, 2002)) But this study is the very first attempt to research on effect of the SCT as a whole. In order to investigate the possible immunoregulatory effects of SCT might have on helper T cell, we isolated CD4 T cell from the splenocytes of the mice and observed the immune function, focusing on the Th1 or Th2 lineage development.

Materials and Methods

1. Mice

Male BALB/c mice at 8 weeks of age were purchased from Samtaco, Korea.

2. Preparation of sample

The Sample (SCT) consists of *Talcum, Rehmannia glutinosa, Angelica sinensis, Paeonia lactiflora, Cnidium officinale, Ledebouriella divaricata, Schizonepeta tenuifolia, Scutellaria baicalensis, Glycyrrhiza uralensis, Mentha arvensis, Cordyceps cicadae* (Table 1). The sample was purchased from KyungHee Medical Center. A total of 240 g was extracted from the sample overnight. The supernatant collected from the extraction was concentrated at 60 ℃ and evaporated in vacuo. 10.1 g (4.21 %) of extract powder was obtained. The sample was dissolved in phosphate buffered saline (PBS) and sterilized by passing through 0.22 -㎛ syringe filter.

3. Cell purification and culture

Splenocytes prepared from BALB/c mice were treated with red blood cell lysing buffer (BD Pharmingen, U.S.A.) CD4 T cells were magnetically isolated by using magnetic microbead-conjugated anti-mouse CD4 mAbs (L3T4) (Miltenyi Germany), as described by the manufacturer. In brief, CD4 T cells were separated by passing the cell suspension over a magnetic-activated cell sorter (MACS) MS+ column held in MACS magnetic separator. The CD4 T cells adhering to the column were cultured in RPMI medium containing 10 % Fetal bovine serum (FBS). Cells were stimulated with or without immobilized anti-CD3/CD28 Ab (Pharmingen, U.S.A.) and incubated for 48 hr at 37 ℃ in 5 % CO_2 with SCT at various concentrations.

Table 1. Contents of *Sopungchungyoung-tang* (SCT)

Contents	Weight
Talcum	30 g
Rehmannia glutinosa	20 g
Angelica sinensis	20 g
Paeonia lactiflora	20 g
Cnidium officinale	20 g
Ledebouriella divaricata	20 g
Schizonepeta tenuifolia	20 g
Scutellaria baicalensis	20 g
Glycyrrhiza uralensis	20 g
Mentha arvensis	20 g
Cordyceps cicadae	30 g
Total amount	240 g

4. Viability and proliferation assay

To measure the viability and proliferation capacity of CD4 T cells in the presence of SCT, cells were placed in a 96-well plate for 48 hr and then treated with the tetrazolium salt,MTS3-(4,5-dimethylthiazol-2-yl)-5-(3carboxymethoxyphenyl) -2H- tetrazolium (Promega, U.S.A.).

5. Analysis of cell surface expression

For cell surface staining, cells were cultured in 24-well plate for 48 hr. Staining of cell surface markers was performed as described by the manufacturer's instruction. In brief, cells were harvested and centrifuged 1000rpm for 5 min. After removing supernatant, the pellet was resuspended in cold wash buffer (PBS / 0.1 %NaN3 / 1 %FBS), centrifuged and resuspended again in 100 ㎕ of wash buffer. Then it was stained with Fluorescein isothiocyanate (FITC)-conjugated CD25 (Pharmingen, U.S.A.) and incubated in the dark for 40 minutes at the temperature of 4 ℃. After two times of thorough washing, the cells were analyzed with a Becton Dickinson FACScan.

6. Th1 / Th2 cell polarization

CD4 T cells were stimulated with anti-CD3/CD28 antibody (Ab) plus recombinant IL-2 (rIL-2) for 3 days. For the condition to be skewed toward Th1, rIL-12 and anti-IL-4 Ab were added, and for skewing condition of Th2, cells were added with rIL-4 and anti-IL-12 Ab.

7. Measurement of cytokine production by CD4 T cells

The levels of IL-4 and IFN-γ in the supernatant of the culture were measured with BD OptEIA Mouse IL-2, IL-4 and IFN-γ set (Pharmingen, U.S.A.) The assayed sensitivities were 200-3.1 pg/ml (IL-2), 2000-31.25pg/ml (IFN-γ) and 500-7.81 pg/ml (IL-4). The plates were read at 570-450 nm and the sample concentrations were determined with the help of the standard curve.

8. Statistical analysis

All the data expressed were mean numbers with an acceptable error range depending on the statistical difference (mean ± S.D.). Independent t-tests were applied in order to determine the statistical differences between the groups.

Results

1. The effect of SCT on the viability and proliferation of CD4 T cells

The viability of CD4 T cells from BALB/c mouse was measured at various concentrations of SCT using the MTS assay. The cells were treated with SCT, its' concentration up to 400 ㎍/㎖, but it did not have any influence on the survival of CD4 T cells (Fig. 1).

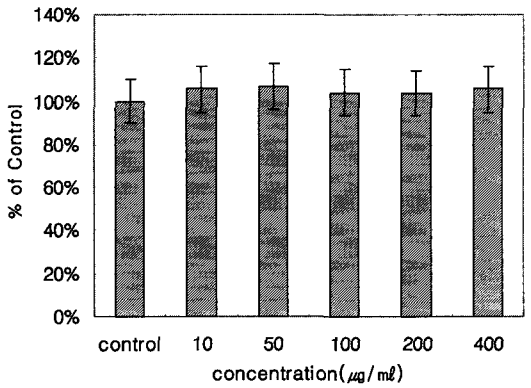

Fig 1. The viability of CD4 Th cells treated with SCT. CD4 Th cells from BALB/c mouse were purified by magnetic cell sorting and cultured in the presence of SCT for 48 hr. Cell survival test was measured using the MTS assay.

However, we did note a distinct decrease of cell proliferation at one point. The cells were treated in the same condition as above, except for an additional stimulation of anti-CD3/CD28. When the concentration level of SCT reached 400 ㎍/㎖, cell proliferation dropped by 49 %. But elsewhere, there was no difference between the SCT treated cells and the control group (Fig. 2).

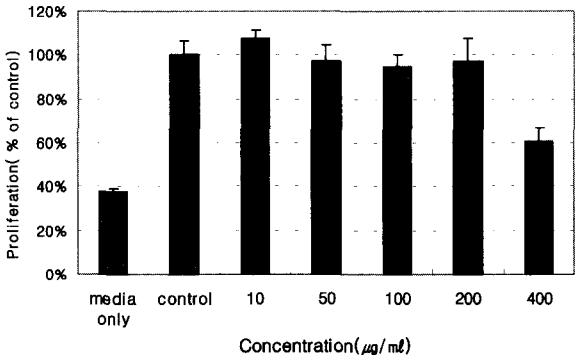

Fig 2. CD4 Th cells were stimulated with anti-CD3/28 in the presence of SCT for 48 hr. Proliferation was measured using the MTS assay

2. The effect of SCT on CD25 expression of CD4 T cells

CD25, which is identified as the alpha chain of the high-affinity IL-2 recepotr, is considered an early activation marker of T cell. So we evaluated the expression of CD25 by flow cytometry, for it can be the evidence of rapid T cell activation. At the treatment level of 10, 50, 100 and 200 ㎍/㎖ of SCT, CD25 expression was slightly increased (Fig.3). Unlike the proliferation induced by SCT, it showed a modest effect on the qualitative response of CD4 T cells, making IL-2 less effective for lymphocyte proliferation.

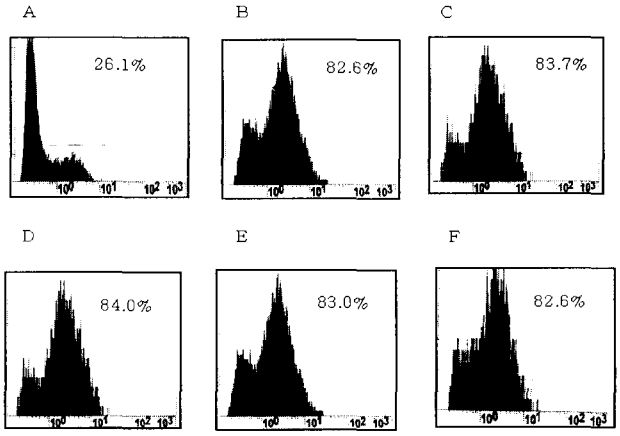

Fig 3. Expression of CD25 on SCT treated CD4 T cells. CD4 Th cells were stimulated with anti-CD3/28 in the presence of SCT. 48 hr later, cells were stained with FITC-conjugated anti-CD25 and were analyzed by flow cytometry. A: cells in media alone. B: cells stimulated with anti-CD3/28. C-F: cells stimulated with anti-CD3/28 in the presence of SCT 10, 50, 100 and 200 ㎍/㎖.

3. The effect of SCT on Th cells' differentiation

Th cell differentiation experiments were performed to examine whether SCT can affect the Th polarization process. CD4 T cells were activated in neutral culture fluid. Cells in Th1-polarized or Th2-polarized conditions were activated in the presence of SCT at concentration of 10, 100 and 200 $\mu g/m\ell$. Supernatants were collected and the production of IFN-γ and IL-4, the signature cytokines for Th1 and Th2 cells, respectively, was measured by an enzyme-linked immunosorbent assay (ELISA).

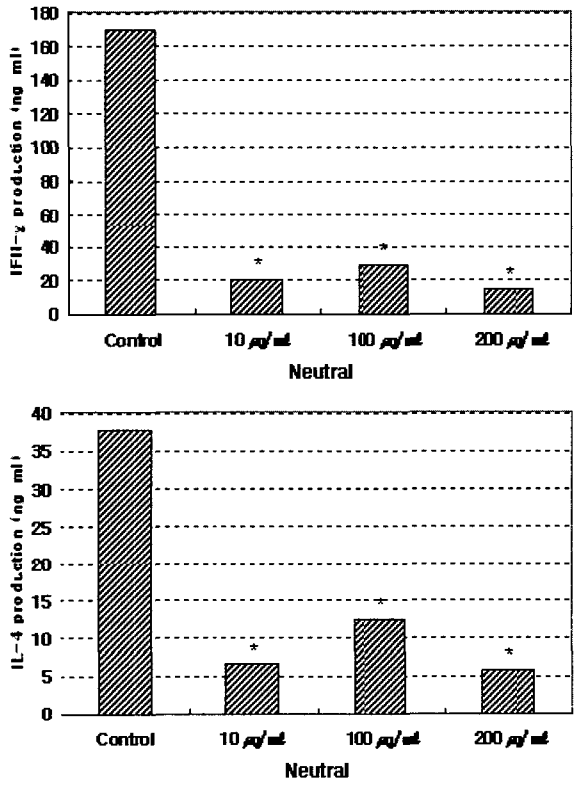

Fig 4. Effect of SCT on Th1 / Th2 cytokine profiles of murine CD4 T cells. CD4 T cells were activated in vitro under neutral condition in the presence of SCT for 72 hr. Cytokine production was measured by ELISA. *: significant at 0.001.

In the case of CD4 T cells under neutral condition where there was only rIL-2 stimulus, SCT had inhibited IFN-γ secretion by 70-80 % (Fig. 4). Likewise, SCT had also inhibited the IL-4 secretion of neutral Th cells by 85-90 %.

The results in polarized conditions were similar to the one in neutral condition above. Inhibitory effects were also observed, but the effect on polarized cells were not a strong as the one on neutral cells. However, the effect between each polarized cells were not identical (Fig. 5).

Whereas under Th1 polarized condition, SCT acted dose-dependently in production of IFN-4, in Th2 cells, the IL-4 production was inversely proportional to the doses of SCT.

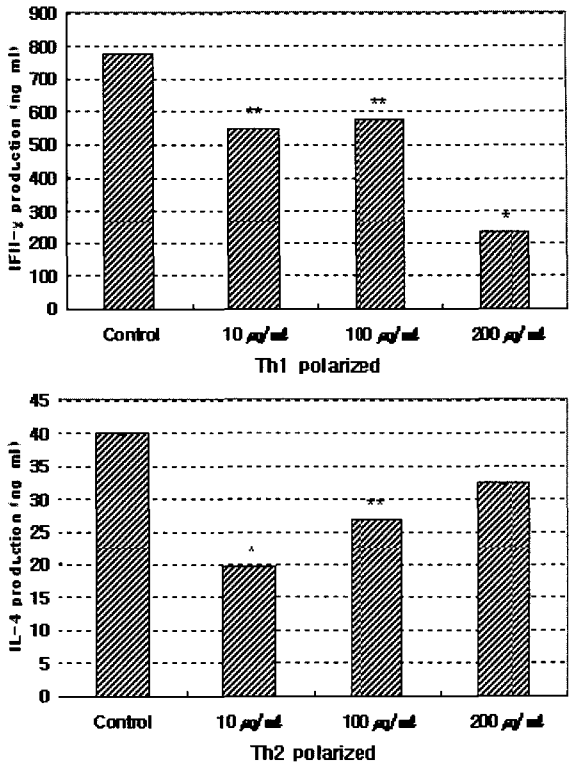

Fig 5. Effect of SCT on Th1 / Th2 cytokine profiles of murine CD4 T cells. CD4 T cells were activated in vitro under Th1 and Th2 polarizing conditions in the presence of SCT for 72 hr. Cytokine production was measured by ELISA. *: significant at 0.001, **: significant at 0.005.

Discussion

The term, "allergy" was first introduced by von Pirquet in 1906. It meant, as Pirquet had recognized, the reactivity changes induced by antigen in both protective immunity and hypersensitivity reactions (Roitt I, 1996). As time passed, meaning of the term was distorted into something a little different. Originally, there are four different mechanisms that can cause allergic responses. But now, only one of them is being frequently used synonymously with the word "allergy", and that is the IgE-mediated allergic disease. One of the best example that describes this IgE-mediated diseases is the term "atopy" (from the Greek atopos, meaning out of place). Persons with atopy have a hereditary predisposition to produce IgE antibodies against common environmental allergens and have one or more atopic diseases. So, on the whole, allergy occurs when a normal immune reaction can not take place.

In a normal immune reaction, the key point is for T cells to recognize the antigen. But the T-cells cannot do this alone. This is where major histocomparibility complex (MHC) performs its' function. Antigen presents antigen-presenting cells (APCs) and combines a part of it to MHC. Then the naive CD4 helper T cells recognize this specific MHC peptide

combinations on the APCs via interactions with the T cell receptor (TCR). This is how an immune reaction would begin.

When begun, which direction the immune reaction will take, in other words, which of the two Th cells (Th1 / Th2) will the precursor cell differentiate into, depends on the cytokine environment present during the priming of the precursor cells. The presence of IL-4 will promote differentiation into Th2 cells, whereas IL-12 and IFN-γ will drive precursor Th cells to differentiate into Th1 cells (Swain SL, 1995).

These two subsets of effector Th cells are then classified and defined on the basis of their distinct cytokine secretion patterns and their immunomodulatory effects. Th1 cells produce inflammatory cytokines, such as tumor necrosis factor β (TNF-β) and IFN-γ, and enhance cellular immunity. Th2 cells produce a different group of cytokines (IL-4, IL-5, IL-6, IL-10 and IL-13) and help B cells secrete antibodies.

The differentiation also has many biological implications, in terms of susceptibility or resistance to a particular disease. For example, Th2-type cytokines such as IL-4, 5, 9, and 13 influence a wide range of events associated with chronic allergic inflammation. IL-4 and IL-13 stimulate the production of IgE and vascular-cell adhesion molecule 1. IL-5 and IL-9 are involved in the development of eosinophils. IL-4 and IL-9 promote the development of mast cells. IL-9 and IL-13 help promote airway hyperresponsiveness (Wills-Karp M, 1998). IL-4, IL-9, and IL-13 promote the overproduction of mucus.

Atopic dermatitis, commonly referred to as eczema, is a chronic skin disorder categorized by scaly and itching rashes. People with eczema often have a family history of allergic conditions like asthma, hay fever, or eczema. Eczema is the most common in infants, and at least half of those cases clear by age 36 months. In adults, it is generally a chronic or recurring condition. A hypersensitivity reaction occurs in the skin, causing chronic inflammation. The inflammation causes the skin to become itchy and scaly. Chronic irritation and scratching can cause the skin to thicken and become leathery-textured.

The symptoms of atopic dermatitis are intense itching, blisters with oozing and crusting, skin redness or inflammation around the blisters, rash (in children under 2 years old, skin lesions begin on the cheeks, elbows, or knees, in adults, the rash more commonly involves the inside surfaces of the knees and elbows), dry, leathery skin areas (more or less pigment than their normal skin tone, located commonly in the inner elbow or behind the knee, may spread to the neck, hands, feet, eyelids, or behind the knee), raw areas of the skin due to scratching, and ear discharges.

According to oriental medicine, the cause of atopic dermatitis has so far been thought of the excess of fire leading to insufficiency of water, which is connected with blood deficiency. Wind syndrome can occur in case of extreme heat. Wind is one of the six pathogenic factors, and it causes disease in association with other pathogenic agents. It is of yang nature and apt to change, resulting in symptoms that are usually migratory and variable.

As dry skin often makes the condition worse, SCT is very useful medicine for that condition. SCT is also an efficacious medicine for nourishing the blood to disperse wind. SCT is a treatment for atopic dermatitis, which is caused by wind as pathogenic factor consumption on account of blood deficiency.

As seen above, Th cells produce cytokine. But sometimes, like in cases of atopic dermatitis for example, the pattern of cytokine production gets abnormal, and it has been suggested that imbalance between the Th1 and Th2 in number and activity may be the cause of it (Yamamoto S, 1997). Acute atopic dermatitis is associated with increased expression of IL-13 (Th2) mRNA. In contrast, there is relative increase in IL-12 mRNA in chronic atopic dermatitis patients suggesting a role for Th1 cells (Hamid Q, 1996; Thepen T, 1997).

The subject of our study was on a treatment that is widely used in Korea for atopic dermatitis discussed above. We studied, and investigated in vitro, the immunological effect of SCT.

In order to find out the effect of SCT on the viability and proliferation of CD4 cells, we measured the viability at various concentrations of SCT using the MTS assay. Up to 400 ㎍/㎖ of SCT, it did not have any influence on the survival of CD4 T cells. However, when cells were activated with anti-CD3/CD28 at the same range of concentration for 48 h, 400㎍/㎖ of SCT decreased cell proliferation by 49 % although elsewhere, there was no difference between the SCT treated cells and control group. This means that SCT does not work as a mitogen to stimulate CD4 T cells.

We also studied about the effect of SCT on Th cells' differentiation. In the case of CD4 T cells under neutral condition where there was only rIL-2 stimulus, SCT inhibited IFN-γ secretion by 70-80 %. Likewise, SCT also inhibited the IL-4 secretion of neutral Th cells by 85-90 %.

In comparison with the neutral condition above, we also experimented with the polarized Th1 cells/ Th2 cells and their production of IFN-γ and IL-4, respectively. The results were similar. There also were inhibitory effects on the polarized cells like there was on neutral cells, they were not as strong on the polarized cells. However, between the Th1 cells and Th2 cells,

the effects of graditional doses of SCT on cytokine production were not identical. Under Th1 polarized condition, SCT acted dose-dependently, while in Th2 cells, the IL-4 production was inversely proportional to the doses of SCT.

Analysis of Th subsets at different intervals after allergen challenges showed that Th2 cells play an important role in initial phase of inflammatory reactions. Whereas later in the stage, Th1 cells can be detected greater in numbers (Bohm I, 1997). Therefore the result that SCT inhibits IFN-γ and IL-4 confirms that it does have a effect on immunoregulation.
From the current study, it can be concluded that SCT exerts inhibitory effects on cytokine production without interfering with immune cells' activity. Further studies are needed to examine how SCT affects cytokine production in a disease-related model.

Conclusion

Sopungchungyoung-tang (SCT) has been widely used in Korea as a treatment of atopic dermatitis. We examined the immunological effect of SCT in vitro. We studied about the effect of SCT on Th cells' differentiation. In the case of CD4 T cells under neutral condition where there was only rIL-2 stimulus, SCT inhibited IFN-γ secretion by 70-80 %. Likewise, SCT also inhibited the IL-4 secretion of neutral Th cells by 85-90 %.

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