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REGULATORY EFFECT OF CYTOKINE PRODUCTION IN ASTHMA PATIENTS BY SOOJI CHIM (KORYO HAND ACUPUNCTURE THERAPY)

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ABSTRACT

Acupuncture has become quite familiar to many Koreans not only for pain, but also for many other health problems, both in acute and chronic conditions. Actually, acupuncture is a therapeutic technique that is part of a larger system of traditional oriental medicine. There are several styles of acupuncture. We investigated the regulatory effects of cytokine production in peripheral blood of asthma patients (AP) by SOOJI CHIM (Koryo Hand Acupuncture Therapy, KHT). Clinical signs of asthma disappeared markedly by KHT. The mean interleukin (IL)-2 and IL-6 plasma levels were lower in the AP group than in the normal group, whereas the mean interferon (IFN)-γ, IL-4, and tumor necrosis factor (TNF)-α levels were higher in the AP group. Plasma IFN-γ and IL-2 levels derived from T helper (Th)1 cells and IL-4 levels derived from Th2 cells were elevated in the AP group by KHT. Especially, plasma IL-6

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levels derived from Th2 cells were elevated significantly in the AP group by KHT. Reduced plasma levels of TNF-α were observed in the AP group by KHT. Plasma IgE levels were also measured but there were no significant differences from each other. During the KHT, there were no other adverse effects. These results indicate that KHT has a good asthma treatment effect, and that its action may be due to the regulation of cytokine production.

INTRODUCTION

SOOJI CHIM, Koryo Hand Acupuncture Therapy (KHT) was developed by Dr. Tae-Woo Yoo in 1971 in Korea. KHT is based on all tenets of traditional body acupuncture as expounded in the classics, but uses a much less invasive treatment technique in which tiny needles are inserted from 1 to 3 mm into points on the hands. The theoretical base of this style of acupuncture rests firmly on the theories of Yin and Yang, the five elements, the 12 viscera and the meridian system of energy flow.[1]

Asthma, which is believed to be associated with genetic components, is one of the most prevalent allergic diseases.[2] The weakened immunity due to air and environment pollution is also an important reason of the continuous increase of asthma patients in these days. Asthma is so prevalent a disease that 7–10% of population in the world is considered to be suffering from this. There is no therapeutic method for a perfect cure, only a few treating agents including bronchodilators and steroid hormone are applied to relieve the symptoms temporally. Asthma is also a kind of hypersensitivity occurred by distorted immune mechanism caused by chronic stimulation. Although the mechanism of asthma is largely unknown, substantial evidence in recent years supports a key role for $CD4^+$/T helper (Th)2 cell-derived cytokines in the pathogenesis of the pulmonary inflammatory response in asthma.[3–8] Th2 lymphocytes produce a panel of cytokines, including interleukin (IL)-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, and GM-CSF.[9] The primary signals that activate Th2 cells are unknown but may be related to the presentation of a restricted panel of antigens in the presence of appropriate cytokines. Interaction of co-stimulatory molecules on the surface of antigen-presenting cells may lead to proliferation of Th2 cells, thus perpetuating mast cell activation and eosinophilic inflammation. This may lead to the production of specific IgE by B lymphocytes under the influence of IL-4, which plays a critical role in the isotype switching of B lymphocytes from IgG to IgE production.[10] Other cytokines, including tumor necrosis factor (TNF)-α and IL-6, may also be important. The IgE produced in asthmatic airways binding to FcεRI on mast cells, priming them for activation by antigen.[4] In contrast, Th1 cells, which synthesize interferon (IFN)-γ and IL-2. Th-1 type cytokines, IFN-γ may play a protective role in countering the IgE-dependent expression

of allergic responses and atopic asthma.[11] Thus, the contemporary viewpoint is that the proasthmatic state reflects an imbalance between Th-1 and Th-2 type cytokine production and action, wherein an induced up-regulated Th2 cytokine response, together which a relatively down-regulated Th1 cytokine response, underlies the cellular and humoral airway inflammatory diathesis in asthma.[12–17]

In the present study, we investigated the changes of Th1 and Th2 responses in patients with asthma by measuring IL-2, IFN-γ derived from Th1 cells and IL-4, IL-6 derived from Th2 cells by KHT. We also measured TNF-α, which is an inflammatory mediator with multiple biologic functions.

MATERIALS AND METHODS

Patients

Blood was obtained from 15 female patients (mean age 43.1, range 14–76) with asthma. Ten healthy adults (5 males and 5 females, mean age 62.5, range 41–68) with no medically diagnosable illness were studied. Informed consent was obtained from all subjects before performing these studies. All samples were collected in a sterile glass tube and allowed to clot spontaneously for 15 min. Plasma was then collected by centrifugation and quickly frozen and stored in aliquots at −80°C until assay.

ELISA of IFN-γ, IL-2, IL-4, IL-6, IgE and TNF-α

Cytokines were measured by a modified ELISA, as described.[18] Sandwich ELISA for IFN-γ, IL-2, IL-4, IL-6, IgE, and TNF-α was carried out in duplicate in 96-well ELISA plates (Nunc, Denmark) coated with each of 100 μL aliquots of mouse anti-human IFN-γ, IL-2, IL-4, IL-6, IgE, and TNF-α monoclonal antibodies (R&D Systems, Minneapolis, MN, USA) at 1.0 μg/mL in PBS at pH 7.4 and was incubated overnight at 4°C. The plates were washed in PBS containing 0.05% Tween-20 (Sigma, St. Lousis, MO, USA) and blocked with PBS containing 1% BSA, 5% sucrose and 0.05% NaN_3 for 1 h. After additional washes, plasma sample or recombinant IFN-γ, IL-2, IL-4, IL-6, IgE, and TNF-α standards were added and incubated at 37°C for 2 h. After 2 h incubation at 37°C, the wells were washed and then each of 0.2 μg/mL of biotinylated anti-human IFN-γ, IL-2, IL-4, IL-6, IgE, and TNF-α were added and again incubated at 37°C for 2 h. After washing the wells, avidin-peroxidase was added and plates were incubated for 20 min at 37°C. Wells were again washed and ABTS substrate (Sigma) was added. Color development was measured at 450 nm using an automated microplate ELISA reader. A standard curve was run on each assay plate using

recombinant human IFN-γ, IL-2, IL-4, IL-6, IgE, and TNF-α (R&D Systems) in serial dilutions.

Total Protein Assay in Plasma

Total protein in plasma was assayed by Bicinchoninic acid protein assay method.

Statistical Analysis

Plasma levels of cytokines between asthma patients (AP) group and KHT group were compared using the paired t-test; Values of cytokines are given in the text as mean ± standard deviation (SD).

RESULTS

When fifteen patients with asthma were treated with KHT, clinical signs of asthma (dry cough, dyspnea and wheezing etc.) disappeared markedly. To study which plasma cytokine levels are changed by KHT, their levels were analyzed in equal amount of total protein.

Plasma IFN-γ Levels

Blood was obtained from 15 female patients with asthma. Higher levels of plasma IFN-γ than healthy subjects (normal controls; 140.5 ± 13.7 pg/mL) were measured in AP. Plasma levels of IFN-γ after KHT were increased (Fig. 1).

Plasma IL-2 Levels

Blood was obtained from 15 female patients with asthma. Lower levels of plasma IL-2 than normal controls (186.9 ± 11.4 pg/mL) were measured in AP. Plasma levels of IL-2 after KHT were increased to three times of AP group (Fig. 2).

Plasma IL-4 Levels

Blood was obtained from 15 female patients with asthma. A slightly increase of plasma IL-4 than normal controls (101.7 ± 17.2 pg/mL) were measured in AP (Fig. 3). Plasma levels of IL-4 after KHT were increased.

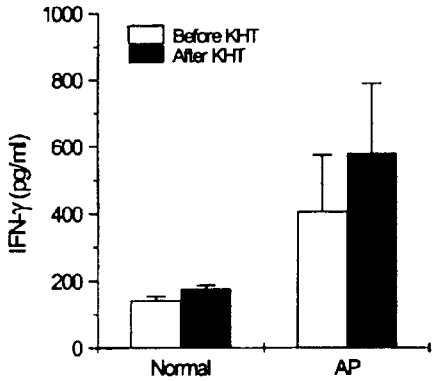

Figure 1. Effect of KHT on plasma IFN-γ level. Healthy subjects (Normal) and AP were treated by KHT for 3 months. Data are shown as mean ± SD. There were no significant differences between before and after KHT treatment.

Plasma IL-6 Levels

Blood was obtained from 15 female patients with asthma. As shown in Fig. 4, we demonstrated that the level of IL-6 was very lower compared with the normal controls (68.7 ± 16.3 pg/mL). However, Plasma levels of IL-6 after KHT were significantly increased to the IL-6 level of normal group (Fig. 4). Our results provide direct evidence that IL-6 levels of plasma were regulated in the AP by KHT.

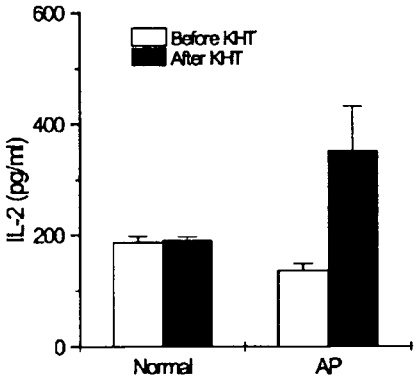

Figure 2. Effect of KHT on plasma IL-2 level. Normal and AP were treated by KHT for 3 months. Data are shown as mean ± SD. There were no significant differences between before and after KHT treatment.

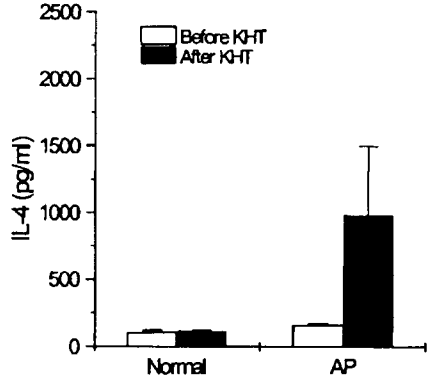

Figure 3. Effect of KHT on plasma IL-4 level. Healthy subjects (Normal) and AP were treated by KHT for 3 months. Data are shown as mean ± SD. There were no significant differences between before and after KHT treatment.

Plasma TNF-α Levels

Blood was obtained from 15 female patients with asthma. Higher levels of plasma TNF-α than normal controls (87.0 ± 10.7 pg/mL) were measured in AP. Plasma levels of TNF-α after KHT were decreased (Fig. 5).

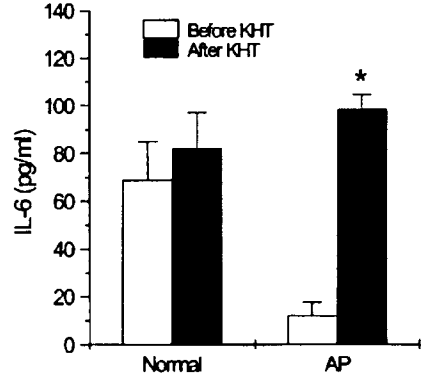

Figure 4. Effect of KHT on plasma IL-6 level. Normal and AP were treated by KHT for 3 months. Data are shown as mean ± SD. * There were significant differences between before and after KHT treatment by paired *t*-test at $P < 0.05$.

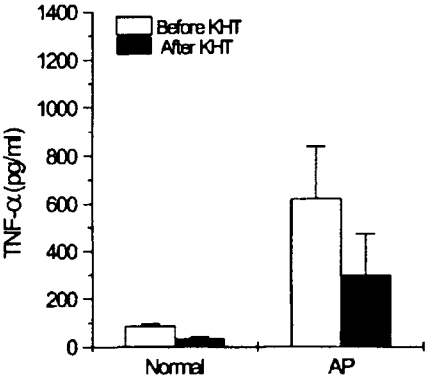

Figure 5. Effect of KHT on plasma TNF-α level. Normal and AP were treated by KHT for 3 months. Data are shown as mean ± SD. There were no significant differences between before and after KHT treatment.

DISCUSSION

In this study, the effect of KHT on the pathogenesis of asthma in TH1/TH2 cytokine level was investigated employing the ELISA method. The ELISA procedure has been widely used for measuring the concentrations of antibodies in human and animal sera due to its simplicity, ease of automation, availability of stable reagents and objective interpretation. We employed the sensitive sandwich ELISA used here specifically to detect plasma cytokines.

We found that IL-2 and IL-6 levels were lower in patients with asthma, whereas IFN-γ, IL-4, and TNF-α levels were higher than in control group. KHT has been shown to increase IFN-γ and IL-2, IL-4 levels. In addition, plasma TNF-α levels were decreased after KHT. IL-6 was significantly increased by KHT.

IL-2 is secreted mainly by antigen or mitogen activated TH1 cells. The biological activities of IL-2 are mediated by a receptor that is almost exclusively expressed on activated T cells but not on resting cells. The binding of IL-2 to its receptor can result in a number of effects, including the induction of proliferation of TH and TC cells and the stimulation of T cells to produce other cytokines such as IFN-γ and IL-4.[19] IFN-γ has important immunoregulatory roles and enhances both antigen specific and non-specific immune responses through actions on monocytes and macrophages.[20–21] The significantly decreased IFN-γ release in the patients with severe asthma confirms a recent study with a small group of patients with severe atopic asthma.[22] But after KHT, these cytokines were increased and clinical signs of AP disappeared markedly. These results suggest that increased IL-2 and

IFN-γ might have beneficial effect in the treatment of AP. The IFN-γ also has been shown to block the effect of IL-4 on B cell.[23–27] The IL-4 has been assessed in a number of disorders associated with increased IgE production. IL-4 levels were elevated in patients with asthma could be related to the high IgE levels found in allergic diseases. However, plasma IgE level was not changed significantly after KHT (data not shown). Thus further elucidation on the roles of IL-4 and IgE should facilitate the development of a novel therapy or preventing maneuver against the asthma.

TNF-α enhances the T-cell mediated response in synergism to antigenic challenge through a direct effect on T cells. Recent studies have also implicated TNF-α as an autocrine growth factor in chronic B-cell malignancies and as relevant mediator in mixed lymphocyte reactions and the early phase of graft-versus-host disease.[28] Therefore, we can expect that reduction of TNF-α by KHT might have beneficial effect in the treatment of AP.

IL-6 is a multifunctional regulator of immune and inflammatory processes that has a range of biologic activities, including important roles in the development of plasma cells and stimulation of the production of acute phase response protein by hepatocytes.[29] Bjorck et al.[30] reported that IL-6 antisense oligonucleotides inhibited IgE production in IL-4 and anti-CD40-stimulated human B cells. Our results show that IL-6 levels of plasma was recovered to the normal control levels by KHT. These results indicate that IL-6 levels of plasma were regulated by KHT and might also play an important role in asthma.

In this report, we show that KHT regulates the imbalance of cytokine levels in AP. Therefore we speculate that KHT may improve immunotherapy and contribute to the development of successful and safe immunotherapy for AP.

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REFERENCES

1. Yoo, T.W. Koryo Hand Acupuncture Therapy. **1999**, *1*, 7.
2. Romagnani, S. Human TH1 and TH2 Subsets: Doubt no more. Immunnol. Today **1991**, *12*, 256.
3. Peter, J.B.; Chung, K. Fan; Clive, P.P. Inflammatory Mediators of Asthma. Pharmacol. Rev. **1998**, *50*, 517.
4. Robinson, D.S.; Hamid, Q.; Ying, S.; Tsicopoulos, A.; Barkans, J.; Bentley, A.M.; Corrigan, C.; Durham, S.R.; Kay, A.B. Predominant Th2-like

Bronchoalbolar T-lymphocyte Population in Atopic Asthma. N. Engl. J. Med. **1992**, *326*, 298.

5. Walker, C.; Bode, E.; Boer, L.; Hansel, T.T.; Blaser, K.; Virchow, J.C. Allergic and Non-allergic Asthmatics have Distinct Patterns of T-cell Activation and Cytokine Production in Peripheral Blood and Bronchoalveloar lavage. Am. Rev. Repir. Dis. **1994**, *146*, 109.
6. Ackerman, V.; Marini, M.; Vittori, E.; Bellini, A.; Vassali, G.; Mattoli, S. Detection of Cytokines and Their Cells Sources in Bronchial Biopsy Specimens from Asthmatic Patients: Relationship to Atopic Status, Symptoms, and Level of Airway Hyperresponsiveness. Chest **1994**, *105*, 687.
7. Corrigan, C.J.; Hamid, Q.; North, J.; Barkans, J.; Moqbel, R.; Durham, S.; Gemou-Engesaeth, V.; Kay, A.B. Peripheral Blood CD4 but not CD8 T-Lymphocytes in Patients with Exacerbation of Asthma Transcribe and Translate Messenger RNA Encoding Cytokines Which Prolong Eosinophil Survival in the Contest of a Th2-type Pattern: Effect of Glucocorticoid Therapy. Am. J. Repir. Cell. Mol. Biol. **1995**, *12*, 567.
8. Humbert, M.S.; Durham, S.R.; Ying, S.; Kimmitt, P.; Barkans, J.; Assoufi, B.; Pfister, R.; Menz, G.; Robinson, D.S.; Kay, A.B. Corrigan, C.J. IL-4 and IL-5 mRNA and Protein in Bronchial Biopsies from Patients with Atopic and non-Atopic Asthma: Evidence Against "Intrinsic" Asthma Being a Distinct Immunopathologic Entity. Am. J. Respir. Crit. Care Med. **1996**, *154*, 1497.
9. Mosmann, T.R.; Cherwinski, H.; Bond, M.W.; Giedlin, M.A.; Coffman, R.L. Two Types of Murine Helper T cell Clone. I. Definition according to Profiles of Lymphokine Activities and secreted proteins. J. Immunol. **1986**, *136*, 2348.
10. Kim, H.M.; Lee, Y.M. Role of TGF-beta 1 on the IgE-Dependent Anaphylaxis Reaction. J. Immunol. **1999**, *162* (8), 4960.
11. Riffo-Vasquez, Y.; Pitchford, S.; Spina, D. Cytokines in Airway Inflammation. Int. J. Biochem. Cell. Biol. **2000**, *32* (8), 833.
12. Lack, G.; Renz, H.; Saloga, J.; Bradley, K.L.; Loader, J.; Leung, D.Y.; Larsen, G.; Gelfand, E.W. Nebulized but not Parenteral IFN-γ Decreases IgE Production and Normalizes Airway Function in a Murine Model of Allergen Sensitization. J. Immunol. **1994**, *152*, 2546.
13. Lack, G.; Gelfan, E.W. The Role of Nebulized IFN-γ in the Modulation of Allergic Responses. In *New Horizons in Allergy Immunotherapy*. A.H. Sehon et al. Eds.; Plenum Press: New York, 1996; 17.
14. Snapper, C.M.; Paul, W.E. Interferon-γ and B-cells Stimulatoryfactor-1 Reciprocally Regulate Ig Isotype Production. Science **1987**, *236*, 944.
15. Coffman, R.L.; Carry, J. A T-cell Activity that Enhances Polyclonal IgE Production and its Inhibition by Interferon-γ. J. Immunol. **1986**, *136*, 949.
16. Pene, J.; Rousset, F.; Briere, F.; Chretien, I.; Bonnefoy, J.Y.; Spits, H.; Yokota, T.; Arai, N.; Arai, K.; Banchereau, J. IgE Production by Normal Human Lymphocytes is Induced by Interleukin 4 and Suppressed by Interferons, and Prostnglandin E2. Proc. Natl. Acad. Sci. U.S.A., **1988**, *85*, 6880.
17. Hessel, E.M. Development of Airway Hyperresponsiveness is Dependent on Interferon-gamma and Independent of Eosinophil Infiltraion. Am. J. Respir. Cell. Mol. Biol. **1987**, *16*, 325.

18. Kim, H.M.; Shin, H.Y.; Jeong, H.J.; An, H.J.; Kim, N.S.; Chae, H.J.; Kim, H.R.; Song, H.J.; Kim, K.Y.; Baek, S.H.; Cho, K.H.; Moon, B.S.; Lee, Y.M. Reduced IL-2 but not Elevated IL-4, IL-6, and IgE Serum Levels in Patients with Cerebral Infarction During the Acute Stage. J. Mol, Neurosci. **2000**, *12*, 191.
19. Paul, B.; Allen, F.R. Expression of Immunoregulatory Cytokines During Acute and Chronic Middle ear Immune Response. Laryngoscope. **1995**, *105*, 629.
20. Peleman, R.; Wu, J.; Fargeas, C.; Delespesse, G. Recombinant Interleukin 4 Suppresses the Production of Interferon Gamma by Human Mononuclear Cells. J. Exp. Med. **1989**, *170*, 1751.
21. Vercelli, D.; Jabara, H.H.; Arai, K.; Geha, R.S. Induction of Human IgE Synthesis Requires Interleukin 4 and T/B Cell Interactions Involving the T Cell Receptor/CD3 Complex and MHC Class II Antigens. J. Exp. Med. **1989**, *169*, 1295.
22. Tang, M.L.; Kemp, A.S.; Thorburn, J.; Hill, D.J. Reduced Interferon-gamma Secretion in Neonates and Subsequent Atopy. Lancet **1994**, *344*, 983.
23. De Vries, J.E.; Gauchat, J.F.; Aversa, G.G.; Punnonen, J.; Gascan, H.; Yssel, H. Regulation of IgE Synthesis by Cytokines. Curr. Opin. Immunol. **1991**, *3*, 851.
24. Thyphronitis, G.; Tsokos, G.C.; June, C.H.; Levine, A.D.; Finkelman, F.D. IgE Secretion by Epstein-Barr Virus-infected Purified Human B Lymphocytes is Stimulated by Interleukin 4 and Suppressed by Interferon Gamma. Proc. Natl. Acad. Sci. U.S.A. **1989**, *86*, 5580.
25. King, C.L.; Gallin, J.I.; Malech, H.L.; Abramson, S.L.; Nutman, T.B. Regulation of Immunoglobulin Production in Hyperimmuno-Globulin E Recurrent-infection Syndrome by Interferon Gamma. Proc. Natl. Acad. Sci. U.S.A. **1989**, *86*, 10085.
26. Chretien, I.; Pene, J.; Briere, F.; De Waal Malefijt, R.; Rousset, F.; De Vries, J.E. Regulation of Human IgE Synthesis. I. Human IgE Synthesis In Vitro is Determined by the Reciprocal Antagonistic Effects of Interleukin 4 and Interferon-gamma. Eur. J. Immunol. **1990**, *20*, 243.
27. Matsumoto, T.; Miike, T.; Yamaguchi, K.; Murakami, M.; Kawabe, T.; Yodoi, J. Plasma Levels of Soluble IL-2 receptor, IL-4 and IgE-binding Factors in Childhood Allergic Diseases. Clin. Experiment. Immunol. **1991**, *85*, 288.
28. McKenzie, J.L.; Calder, V.L.; Starling, G.C.; Hart, D.N. Role of Tumour Necrosis Factor-alpha in Dendritic Cell-Mediated Primary Mixed Leucocyte Reactions. Bone Marrow Transplant. **1995**, *15*, 163.
29. Gauldie, J.; Richards, C.; Harnish, D.; Lansdorp, P.; Baumann, H. Interferon beta 2/B-cell Stimulatory Factor Type 2 Shares Identity with Monocyte-derived Hepatocyte-stimulating Factor and Regulates the Major Acute Phase Protein Response in Liver Cells. Proc. Natl. Acad. Sci. U.S.A. **1987**, *84*, 7251.
30. Bjorck, P.; Larsson, S.; Andang, M.; Ahrlund-Richter, L.; Paulie, S. IL-6 Antisense Oligonucleotides Inhibit IgE Production in IL-4 and Anti-CD40-Stimulated Human B-Lymphocytes. Immunol. Lett. **1998**, *61*, 1.