

Suppression of TH2-Type Immune Response-Mediated Allergic Diarrhea Following Oral Administration of Traditional Korean Medicine: *Atractylodes Macrocephala* Koidz

Sae-Hae Kim,¹ Ha-Na Jung,¹ Kyung-Yeol Lee,¹ Ju Kim,²
Jeong-Chae Lee,³ and Yong-Suk Jang^{1,2}

¹Division of Biological Sciences and Research Center of Bioactive Materials, Chonbuk National University, Chonju, Korea

²Bank for Cytokine Research, Chonbuk National University, Chonju, Korea

³Laboratory of Cell Biology in Department of Orthodontics and Institute of Oral Bioscience, Chonbuk National University, Chonju, Korea

Atractylodes macrocephala Koidz (AMK) is well-known as a digestive and tonic material and is widely used in traditional Korean herbal medicines. Previously, we found that protein samples obtained from the medicines could induce a preferential stimulation of type 1, rather than type 2, helper T lymphocytes (Th) immune responses in vitro. Since immune response induction is controlled by the balanced activation between Th1- and Th2-type immune responses, we tested to see whether or not the AMK protein sample could inhibit the ovalbumin (OVA)-mediated allergic diarrhea, whose induction has been known to be mediated by the Th2-type immune responses. The sample treatment markedly stimulated lymphocyte proliferation, antibody production, and cytokine secretion in vitro, showing a preferential stimulation of Th1-type immune responses. In particular, oral administration of the AMK sample suppressed the OVA-mediated allergic diarrhea in mice. The sample treatment also

Address correspondence to Yong-Suk Jang, Ph.D., Division of Biological Sciences and the Institute for Molecular Biology and Genetics, Chonbuk National University, Chonju 561-756, Korea; Fax: 82-63-270-4312; E-mail: yongsuk@moak.chonbuk.ac.kr

suppressed the OVA-mediated enhanced levels of total immunoglobulin (Ig) E, as well as OVA-specific IgE, which are closely associated with Th2 cell stimulation in mice. Furthermore, the oral treatment of the sample significantly increased gamma interferon (IFN- γ) production by lymphocytes, isolated from spleen and large intestine of the mice, that had been systematically challenged with OVA. Consequently, the oral administration of AMK protein sample suppressed the OVA-mediated allergic diarrhea by preferential stimulation of the Th1-type immune responses.

Keywords *Atractylodes macrocephala* Koidz, Immune modulation, Oral administration, OVA-mediated allergic diarrhea.

INTRODUCTION

Allergies are one of the hypersensitivity diseases, and the degree of the diseases varies depending on the types of allergy involved. Atopic diseases and nasal asthma are typical type I hypersensitivity reactions, that are mediated by the immunoglobulin (Ig) E antibody in humans.^[1] In addition, diarrhea induced by ingested food allergens is characterized by an imbalanced ion exchange and high levels of serum IgE antibodies.^[2] Therefore, we believe that the IgE antibody plays an important role in hypersensitivity diseases.

Immunologically, the helper T lymphocytes (Th)1/Th2 cytokines interact reciprocally to maintain a balanced immune network.^[3–5] Immune response deviation toward either the Th1 or Th2 pathway can elicit a number of pathologic conditions, such as skewed stimulation of Th2 responses in allergic reactions and skewed stimulation of Th1 deviation in organ-specific autoimmunity. In general, the Th2 cells produce predominantly interleukins (IL)-4, IL-5, IL-10, and IL-13, and are commonly associated with strong antibody and allergic immune responses, including IgE-dependent mast cell degranulation and eosinophil infiltration into inflammation sites.^[6,7] In contrast, Th1 cells are involved in cell-mediated immune reactions and produce IL-2 and gamma interferon (IFN- γ), which inhibit eosinophil infiltration and IgE and IgG1 secretion and enhance IgG2a secretion in vivo.^[5,6,8] Hence, selective priming of the Th1/Th2 balance, namely shifting the balance from Th2 to Th1 dominance, is thought to be an ideal strategy for treating allergic diseases.

Traditional herbal medicines have long been used to treat a variety of diseases and to maintain good health. Among the traditional herbal medicines, *Atractylodes macrocephala* Koidz (AMK) is widely used in digestive and tonic materials in traditional Oriental medicines.^[9] Recently, there has been increasing interest in studying the pharmacomedicinal roles of this drug, and its beneficial effects have been reported.^[10] In the previous study, we showed that oral administration of the traditional Korean medicines, Soamsan and Bo-yang-hwan-o-tang, in which AMK is one of the main components,

stimulated antigen-specific immune responses by selectively priming specific subtypes of the helper T cell populations. The stimulated activity of the medicine was associated closely with glycoprotein levels.^[11,12] In addition, we found that AMK contains a potential ability to stimulate immune responses and its activity was associated with glycoprotein(s) with molecular weights of around 30 kDa.

Therefore, we prepared the protein sample containing mainly 30 kDa glycoproteins from origin sample of AMK water extract. Initially, we examined whether the sample could induce the selective and preferential stimulation of Th1-type rather than Th2-type immune responses in vitro. Since immune response induction may be controlled by the balanced activation between Th1- and Th2-type immune responses, we also tested if the oral administration of the sample could inhibit the ovalbumin(OVA)-mediated allergic diarrhea, whose induction was known to be mediated by Th2-type immune responses.^[13,14]

MATERIALS AND METHODS

Chemicals, Plastics, and Mice

Unless otherwise specified, all chemicals and plastics used in this study were purchased from Sigma Chemical (St. Louis, MO, USA) and Falcon Labware (Becton-Dickinson, Franklin Lakes, NJ, USA), respectively. Cytokine reagents were obtained from BD Biosciences PharMingen Inc. The 4- to 5-week-old BALB/c mice (haplotype: H-2^d), from Orient Co. (Seoul, Korea), were housed 5 mice per cage following the standard guidance of laboratory animals during experimental periods.

Preparation of the AMK Sample

AMK was generously provided by Dr. W.-H. Woo (College of Oriental Medicine, Wonkwang University, Iksan, Korea) and authenticated by botanists at the Korea Institute of Oriental Medicine. Initially, to prepare a water extract of AMK, air-dried AMK (100 g) was cut into small pieces, mixed with 1,000 ml of distilled water in a screw-capped flask, and then shaken at 4°C for 3 days. The extract was subsequently filtered through filter paper (Whatman No. 3) and centrifuged at $5,000 \times g$ for 10 min to remove debris. Collected supernatants were lyophilized to produce the original sample of 10.2 g, which was 10.2% of the initial amount. Then 1 g of the sample was dissolved with 10 ml of 80% (NH₄)₂SO₄ and centrifuged to collect the protein precipitate. The precipitate was then dialyzed and lyophilized to yield 72 mg of the protein sample, which consisted mainly of 30 kDa glycoprotein (Fig. 1A). The samples were stored at -20°C before use.

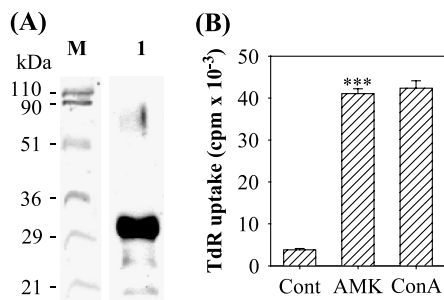


Figure 1: SDS-PAGE analysis of protein sample of AMK. (A) Protein sample of AMK was loaded 200 μ g in a lane, electrophoresed, and stained with CBB (lane 1). M represents a molecular weight marker. (B) BALB/c spleen cells (2×10^6 cells/ml) were incubated with the 10 μ g/ml AMK protein sample for 48 hr and labeled with (methyl-³H) TdR for the last 16 hr. Con A was a positive control. The results are expressed as the mean tritiated-thymidine uptake in cpm $\pm$ SE in triplicate cultures. The same experiments were repeated three times, and the table shows a representative result. *** $p < 0.001$ indicate a significant difference between the experimental and control values.

In Vitro Assays

To assess the effects of the AMK protein sample on the immunoglobulin (Ig) content, cytokine production, and lymphocyte proliferation, by the immune cells in vitro, splenocytes (2×10^6 cells/ml) maintained in 96-well tissue culture plates were exposed to the sample for 48 hr. The amount of the total IgG and IgG2a subtype and the levels of IFN- γ and IL-2 were determined by ELISA, and the level of lymphocyte proliferation was assessed through a tritiated-thymidine uptake assay as described previously.^[15]

Antigen Challenge and Application of the AMK Sample

Antigen challenge was performed according to the methods of Kweon et al.^[13] Mice were initially primed systemically, once with 1 mg/head of OVA (fraction V) emulsified in 100 μ l of complete Freund's adjuvant (CFA; DIFCO Laboratories, Detroit, MI, USA). Then, 1 week after the systemic priming, mice were repeatedly challenged with 50 mg/head of OVA dissolved in 300 μ l of PBS, by gastric intubation every other day for 3 weeks. Next, 1 day after the antigen challenge, the mice were orally administered with 1 mg/head of AMK sample every other day during the OVA treatment. For the control, mice were repeatedly given 50 mg/head of OVA in PBS by gastric intubation, without any systemic challenge. Then 2 hr after the last oral administration of the AMK sample or the antigen challenge, the mice were sacrificed and OVA-induced allergic reactions were analyzed.

Total and OVA-Specific IgE Antibodies in Serum

Levels of the total and OVA-specific IgE antibodies in the mice sera were determined by ELISA, as described previously.^[13] Briefly, each well of the ELISA plate was coated with purified rat antimouse IgE mAb (BD Biosciences PharMingen) and incubated overnight at 4°C. After blocking them with 3% BSA in PBS, serial dilutions (50 µl) of the serum samples were added to each well and incubated for 2 hr at 37°C. After extensive washing, biotinylated rat antimouse IgE mAb (BD Biosciences PharMingen) for total IgE Ab or biotinylated OVA for OVA-specific IgE Ab were added, respectively. Next, the plates were incubated overnight at 4°C, and peroxidase-labeled antibiotin mAb (Vector Laboratories, Burlingame, CA, USA) for total IgE, or 10 ng/ml AquaLite streptavidin (SeaLite Sciences, Notcross, Sweden) in 2 mM EGTA in PBS for OVA-specific IgE were added, respectively. After washing, the substrate was added to develop the color, and the absorbance was measured at 405 nm, using a SpectraCount™ (Packard Instrument, Downers Grove, IL, USA) ELISA reader.

Isolation of Mononuclear Cells

At 2 hr after the last oral administration of the AMK sample, mice were sacrificed and their spleens were removed aseptically. Spleen cell populations were prepared using RPMI-1640 medium, supplemented with antibiotics and 10% FBS (HyClone, Logan, UT, USA). One million spleen cells per milliliter were resuspended in 500 µl media to spread onto 24-well flat-bottomed plates for cytokine analysis. In addition, mononuclear cells were dissociated from the large intestine using a collagenase (type IV; 0.5 mg/ml of RPMI 1640), after the removal of colonic patches.^[16] The single-cell suspensions were then pooled and further purified using a Percoll gradient, as described previously.^[17]

Measurement of OVA-Specific Cytokine Production

Cytokine levels of culture supernatants of OVA-stimulated mononuclear cells, isolated from the spleen and large intestine, were measured by ELISA, which was serviced by the Bank for Cytokine Research (Chonbuk National University, Chonju, Korea). Briefly, the mononuclear cells were stimulated in the 24-well flat-bottom plates with 100 µg/ml of OVA for 3 days. Culture supernatants were then collected and processed for ELISA using the IFN-γ- and IL-4-specific OptiEIA™ kit (BD PharMingen). Cytokine levels were calculated based on the standard curves generated using known concentrations of recombinant cytokine proteins.

Statistical Analyses

All the results were expressed as the mean $\pm$ standard error (SE). A one-way ANOVA, using SPSS v 10.0 software, was used to make a statistical comparison among the groups. A value of $p < 0.05$ was considered significant.

RESULTS

AMK Sample Treatment

To investigate the effects of the AMK protein sample on immune stimulating activity *in vitro*, titers of the total IgG and IgG2a, levels of IFN- γ and IL-4, and tritiated-thymidine incorporation in splenocytes were measured (Figs. 1–3). As shown in Fig. 1B, the protein sample treatment markedly stimulated the splenocytes proliferation, such that when the splenocytes were treated with 10 $\mu\text{g/ml}$ of the sample, the tritiated-thymidine uptake was 10.67-fold higher than that obtained from untreated control cells. Treatment of the AMK sample also significantly increased the titers of total IgG (Fig. 2A). This increase correlated with the augmented level of IgG2a (Fig. 2B), which was known to be closely related to the level of IFN- γ .

As expected, the AMK sample significantly increased the production of IFN- γ , compared with the control. For example, 10 $\mu\text{g/ml}$ of the sample treatment stimulated the production of a 64.42-fold higher level of IFN- γ by splenocytes than by untreated control splenocytes (0.72 ng/ml), and the level was statistically similar to that obtained after treating splenocytes with Con A (42.63 ng/ml) (Fig. 3A). Similarly, a prominent increase in IL-2 also was observed by treating splenocytes with the sample (Fig. 3B). However, no

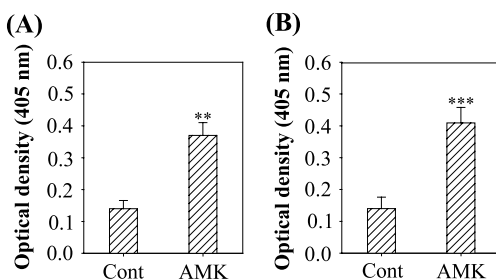


Figure 2: Effects of the protein sample of AMK on IgG production by mouse splenocyte. The levels of total IgG (A) and IgG2a (B) in the culture supernatants of splenocytes treated with the AMK protein sample were determined by ELISA, as described in the Materials and Methods section. The results represent values obtained with culture supernatants 64-fold diluted and are expressed as the mean $\pm$ SE. The same experiments were repeated three times. The figure shows a representative result. ** $p < 0.01$ and *** $p < 0.001$ indicate significant differences between the values of the protein sample and the control.

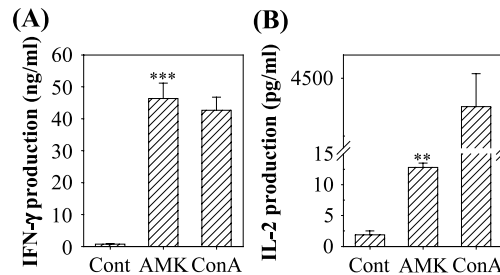


Figure 3: Effects of the AMK protein sample treatment on cytokine production by mouse splenocytes. Splenocytes were cultured and exposed to 10 μ g/ml of the protein sample for 48 hr, and the levels of IFN- γ (A) and IL-2 (B) were analyzed by ELISA. The same experiments were repeated three times. The figure shows a representative result expressed as the mean $\pm$ SE. Con A was used as positive control. ** p < 0.01 and *** p < 0.001 indicate a significant difference between the experimental and control values.

significant changes were detected in the levels of IL-4 and IL-5 after stimulating splenocytes with the sample (data not shown).

OVA-Mediated Allergic Diarrhea

To test the effects of the AMK sample on immune modulating activity *in vivo*, we initially tried to establish the model mice that undergo an OVA-mediated allergic diarrhea, which has been suggested to be caused by the enhanced Th2 immune responses, as described in the Material and Methods section. We found that repeated oral administration of 50 mg/head of OVA to BALB/c mice after previous subcutaneous immunization with OVA in CFA resulted in acute allergic diarrhea (Fig. 4A and 4B). OVA-induced diarrhea started after the seventh gastric intubation of the antigen to the systemically primed mice (Fig. 4A). However, repeated oral challenge of OVA without systemic priming did not induce any significant clinical sign of diarrhea. In addition, diarrhea was observed by direct observation of the colon of the systemically primed mice (Fig. 4B). As shown in the figure, the liquid stool observed following OVA-induced diarrhea was not seen in the colon of the nonimmunized mice, and the nonimmunized mice showed the solid pellets in the colon.

Total IgE and IgG1 Antibodies Dramatically Enhanced

To estimate the changes of IgE and IgG1 production by the allergen challenge, we examined serum levels of the total IgE and IgG1. Mice that received subcutaneous immunization of the OVA before oral challenge produced high levels of IgE, compared with that of nonsystemically primed mice, which were immunized with PBS instead of OVA (Fig. 5A). In addition,

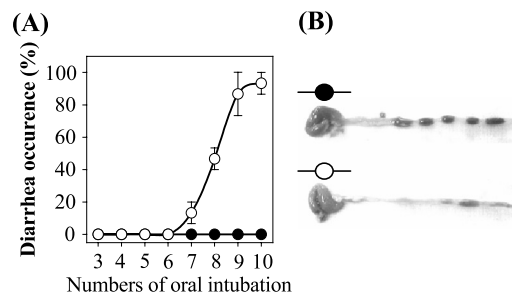


Figure 4: Induction of ovalbumin(OVA)-induced allergic diarrhea. (A) Diarrhea occurrence was assessed 1 hr after the oral challenge of 50 mg/head OVA to the mice that were systemically primed with OVA (open circle), or injected with PBS instead of the antigen (closed circle). (B) After the 10th oral challenge of 50 mg/head OVA to the mice, the mice were sacrificed and their colons were photographed.

the IgG1 antibody level, which is closely related to the primed stimulation of Th2-type T lymphocytes, was significantly higher in the serum of mice that received systemic OVA immunization than in those that received only oral administration of OVA (Fig. 5B).

Oral Administration of AMK Sample

To investigate the effects of the AMK sample on OVA-induced allergic diarrhea, sera were collected from BALB/c mice primed and orally challenged with OVA as described in the Materials and Methods section. The increased levels of total and OVA-specific IgE were significantly reduced in sera from the systemically primed mice fed AMK sample (Fig. 6A and 6B). For example, amount of the total IgE antibody in the sera of the systemically primed mice

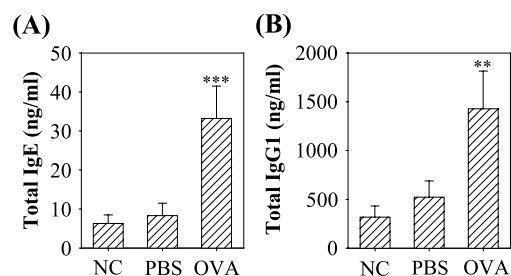


Figure 5: Effect of the oral challenge of OVA on the levels of total serum IgE and IgG1 contents. The mice that were subcutaneous immunized, either with OVA (systemic priming) or with PBS (control), were repeatedly given 50 mg/head of OVA in PBS by gastric intubation. Then 2 hr after the last oral challenge of the antigen, levels of total IgE (A) and IgG1 antibodies (B) in the mice sera were determined by ELISA, as described in the Materials and Methods section. The same experiments were repeated three times, and the figure shows a representative result. *** $p < 0.001$ indicates significant difference between the experimental and control values.

was elevated to a 4.1-fold higher level compared with the level of the control mice (8.1 ng/ml). Meanwhile, while this increased level of the total IgE antibody was reduced to 13.9 ng/ml by the oral treatment of AMK protein sample (1 mg/head). To determine if the reduced levels of the total and OVA-specific IgE antibodies observed in sera of the systemically primed mice following oral administration of the AMK sample were related to the inhibition of OVA-induced diarrhea, the colons of mice were removed 2 hr after the last oral administration of AMK sample or antigen challenge. This process revealed that the liquid stool formation observed in the colon of the systemically primed mice was clearly reduced by oral administration of the AMK sample (Fig. 6C).

To understand the nature of the inhibition of OVA-induced diarrhea following oral administration of the AMK protein sample, we measured the levels of IFN- γ and IL-4 using the mononuclear cells dissociated from the spleen and large intestine of the mice. After antigen stimulation, IFN- γ levels were higher in the mice fed AMK sample than in those primed only systemically (Fig. 7). When splenic and intestinal mononuclear cells, prepared from systemically primed mice fed 1 mg/head of the AMK sample, were restimulated with 100 μ g/ml of OVA for 3 days, 11.4 and 3.8 ng/ml of IFN- γ , respectively, were produced. In contrast, the cells from the systemic primed mice only produced 6.8 and 1.3 ng/ml in the splenic and intestinal mononuclear

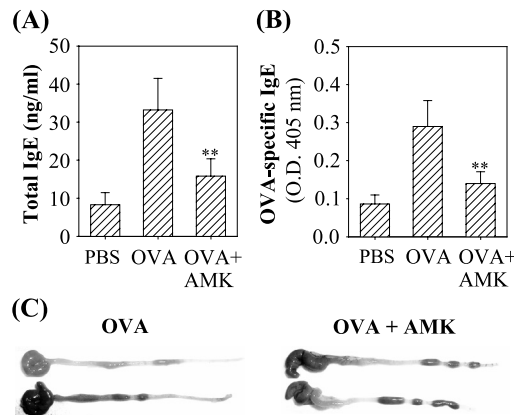


Figure 6: Effects of the AMK sample on total and OVA-specific IgE antibodies in the serum. The systemically primed mice were repeatedly given 50 mg/head of OVA in PBS by gastric intubation, either with or without oral administration of 1 mg/head AMK sample. Then 2 hr after the 10th oral challenge, or oral administration of the antigen and AMK sample, respectively, the levels of total (A) and OVA-specific IgE antibodies (B) in the mice sera were determined by ELISA, as described in the Materials and Methods section. (C) A representative photograph of the mice colon 2 hr after the 10th oral challenge of OVA, or AMK sample (left and light two samples, respectively). The same experiments were repeated three times, and the figures are a representative result. *** $p < 0.001$ indicates a significant difference between the experimental and control values.

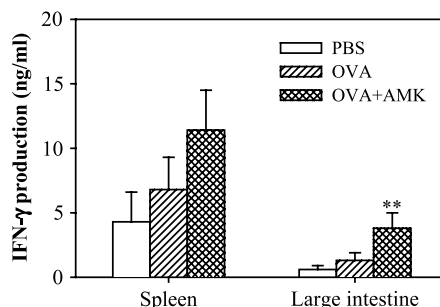


Figure 7: Effect of the AMK sample on IFN- γ production after antigen stimulation of mononuclear cells. The mononuclear cells prepared from the spleen and large intestine of the control, or diarrhea induced mice, were cultured in the presence of 100 μ g/ml of OVA for 3 days. Then the culture supernatants were analyzed by the cytokine-specific ELISA. The result is expressed as the mean $\pm$ SE. The same experiments were repeated three times, and the figure shows a representative result. ** $p < 0.01$ represents a significant difference between the experimental and control values.

cells, respectively. However, IL-4 expression by the OVA-stimulated mononuclear cells was not significantly different between the samples prepared from systemically primed mice with and without the oral administration of the AMK sample (data not shown).

DISCUSSION

Initially, we found that the AMK protein sample significantly increased the levels of total IgG and IgG2a secretion, IFN- γ production, and tritiated-thymidine uptake by splenocytes (Figs. 1–3). These results suggested that AMK could induce a marked stimulation of Th1-type specific immune responses. Since we believed that the immune response induction is controlled by the balanced activation between Th1- and Th2-type immune responses,^[3–5] we postulated that enhanced stimulation of Th1-type immune responses by the AMK sample treatment could inhibit the Th2-type immune responses. As an initial attempt to confirm this assumption, we tested if the oral administration of the medicine could inhibit the OVA-induced allergic diarrhea, whose induction was known to be mediated by Th2-type immune responses.^[13,14]

Consistent with the previous report,^[13] OVA-mediated allergic diarrhea and the increases of total IgE and IgG1 antibodies were observed only in the systemically primed mice compared with the mice that only with oral administration without systematic priming (Figs. 4 and 5). These results clearly supported that the induction of OVA-mediated allergic diarrhea was mediated by the Th2-type immune responses. Interestingly however, oral administration of the AMK sample to systemically primed mice significantly reduced the levels of total and OVA-specific IgE antibodies in serum and also

inhibited acute diarrhea (Fig. 6). Similar to the reduction of IgE antibodies, the level of IgG1 antibody also was decreased in the serum of immunized mice by the AMK sample treatment (data not shown). These results indicated that oral treatment of the AMK protein sample enhanced the induction of the Th1-type specific immune response to the allergic antigen. This indication was further supported by the result where levels of IFN- γ in the mononuclear cells, dissociated from spleen and large intestine of systemically primed mice, were significantly increased by oral administration of the AMK sample (Fig. 7). Therefore, we postulated that oral administration of the AMK sample selectively stimulated Th1 cells rather than Th2 cells by increased IFN- γ versus IL-4 and was likely to play an important role in alleviating OVA-specific immune responses.

Allergies are one of the common problems in developed countries. The IgE antibody is known to be related closely to allergic responses, which are considered to be due to the development and activation of Th2 cells.^[2,6,7] Therefore, application of the method of selectively priming the Th1-type immune response could be a rational strategy for treating allergic diseases.^[18–20] The present study demonstrated that oral administration of the AMK sample potentially enhanced Th1-mediated or, alternatively, suppressed Th2-mediated, immune responses. We assume that the AMK-mediated modulation of OVA-mediated immune responses might be closely involved in STAT6 signaling, which was found to play an essential role in the development of OVA-induced allergic diarrhea, although the direct evidence is under investigation.^[13]

Collectively, we speculate that AMK may be used for immunotherapy in patients with skewed immune responses of Th2-type T cells, such as bronchial and nasal asthma, and allergic diseases. However, further detailed investigations are required to determine the exact mechanism(s) involved in the AMK sample-mediated inhibition of OVA-induced allergic diarrhea. Additional experiments also should be carried out to elucidate the active components responsible for the antiallergic response mediated by the AMK sample and to identify the physicochemical property of the active compounds.

ACKNOWLEDGMENTS

This work was supported by the grant from Ministry of Health and Welfare, 03-PJ1-PG3-22000-0001, to Dr. J.-C. Lee. Dr. K.-Y. Lee was supported by the Chonbuk National University Post-Doctoral Fellowship Program.

REFERENCES

1. Serafin, W.E.; Austen, K.F. Mediators of immediate hypersensitivity reactions. *N. Engl. J. Med.* **1987**, *317*, 30–34.

2. Romagnani, S. Atopic allergy and other hypersensitivities editorial overview: technological advances and new insights into pathogenesis prelude novel therapeutic strategies. *Curr. Opin. Immunol.* **1995**, *7*, 745–750.
3. Parronchi, P.; De Carli, M.; Manetti, R.; Simonelli, C.; Sampognaro, S.; Piccinini, M.P.; Macchia, D.; Maggi, E.; Del Prete, G.; Romagnani, S. IL-4 and IFN (alpha and gamma) exert opposite regulatory effects on the development of cutolytic potential by Th1 or Th2 human T cell clones. *J. Immunol.* **1992**, *149*, 2977–2983.
4. Spellberg, B.; Edwards, J.E. Type 1/type 2 immunity in infectious diseases. *Clin. Infect. Dis.* **2001**, *32*, 76–102.
5. Carter, L.L.; Dutton, R.W. Type 1 and type 2: a fundamental dichotomy for all T cell subsets. *Curr. Opin. Immunol.* **1996**, *8*, 336–342.
6. 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*, 2348–2357.
7. Cerwenka, A.; Carter, L.L.; Reome, J.B.; Swain, S.L.; Dutton, R.W. In vivo persistence of CD8 polarized T cell subsets producing type 1 or type 2 cytokines. *J. Immunol.* **1998**, *161*, 97–105.
8. Kapsenberg, M.L.; Wierenga, E.A.; Bos, J.D.; Jansen, H.M. Functional subsets of allergen-reactive human CD4+ T cells. *Immunol. Today* **1991**, *12*, 392–395.
9. Yim, D.S.; Yu, S.C.; Chi, H.J. Phytochemical study on the rhizome of *Atractylodes japonica* from Korea (Korean). *Korea J. Pharm.* **1988**, *19*, 228–232.
10. Park, J.S.; Kim, Y.S.; Lee, J.W.; Park, R.G.; Jeon, B.H.; Woo, W.H.; Jeong, W.Y. Effect of rhizoma *Atractylodis macrocephalae* on the melanogenesis (Korean). *Korea J. Orient. Med. Pathol.* **1999**, *13*, 91–98.
11. Kim, E.H.; Lee, J.C.; Kim, J.; Son, Y.O.; Jung, G.H.; Jang, Y.S. Modulation of antigen-specific immune responses by the oral administration of a traditional medicine, Bo-yang-hwan-o-tang. *Immunopharmacol. Immunotoxicol.* **2002**, *24*, 423–440.
12. Kim, E.H.; Lee, J.C.; Park, C.H.; Lee, M.Y.; Jang, Y.S. Soamsan, a traditional Korean medicine, enhances antigen-specific immune responses in low-responder mice via the combined activity of glycoproteins and endotoxins. *Int. Immunopharmacol.* **2002**, *2*, 1529–1540.
13. Kweon, M.N.; Yamamoto, M.; Kajiki, M.; Takahashi, I.; Kiyono, H. Systemically derived large intestinal CD4(+) Th2 cells play a central role in STAT6-mediated allergic diarrhea. *J. Clin. Invest.* **2000**, *106*, 199–206.
14. Brandt, E.B.; Strait, R.T.; Hershko, D.; Wang, Q.; Muntel, E.E.; Scribner, T.A.; Zimmermann, N.; Finkelman, F.D.; Rothenberg, M.E. Mast cells are required for experimental oral allergen-induced diarrhea. *J. Clin. Invest.* **2003**, *112*, 1666–1677.
15. Lee, J.C.; Jung, H.N.; Kim, J.; Woo, W.H.; Jeong, W.Y.; Chung, G.H.; Jang, Y.S. Selective priming of Th1-mediated antigen-specific immune responses following oral administration of mixed prescriptions of traditional Korean medicines. *Clin. Chim. Acta* **2003**, *329*, 133–142.
16. Fujihashi, K.; McGhee, J.R.; Kweon, M.N.; Cooper, M.D.; Tonegawa, S.; Takahashi, I.; Hiroi, T.; Mestecky, J.; Kiyono, H. Gamma/delta T cell-deficient mice have impaired mucosal immunoglobulin A responses. *J. Exp. Med.* **1996**, *183*, 1929–1935.

17. Kweon, M.N.; Fujihashi, K.; VanCott, J.L.; Higuchi, K.; Yamamoto, M.; McGhee, J.R.; Kiyono, H. Lack of orally induced systemic unresponsiveness in IFN-gamma knockout mice. *J. Immunol.* **1998**, *160*, 1687–1693.
18. Hsu, H.Y.; Lin, B.F.; Lin, J.Y.; Kuo, C.C.; Chiang, W. Suppression of allergic reactions by dehulled adlay in association with the balance of TH1/TH2 cell responses. *J. Agric. Food Chem.* **2003**, *51*, 3763–3769.
19. Zhu, F.G.; Kandimalla, E.R.; Yu, D.; Tang, J.X.; Agrawal, S. Modulation of ovalbumin-induced Th2 responses by second-generation immunomodulatory oligonucleotides in mice. *Int. Immunopharmacol.* **2004**, *4*, 851–862.
20. Tanaka, A.; Ohashi, Y.; Kakinoki, Y.; Nakai, Y. Immunotherapy suppresses both Th1 and Th2 responses by allergen stimulation, but suppression of the Th2 response is a more important mechanism related to the clinical efficacy of immunotherapy for perennial allergic rhinitis. *Scand. J. Immunol.* **1998**, *48*, 201–211.