

Selective priming of Th1-mediated antigen-specific immune responses following oral administration of mixed prescriptions of traditional Korean medicines

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Abstract

Background: In previous studies, we showed that oral administration of traditional Korean medicines, Soamsan (SA) and Bo-yang-hwan-o-tang (BHT), modulated antigen-specific immune responses in mice. **Methods:** We attempted to strengthen cell-mediated immune responses in mice using two mixed prescriptions composed mainly of components used in SA and/or BHT. The effect of oral administration of the medicines on the induction of antigen-specific immune responses was investigated using hen egg-white lysozyme (HEL) as a model antigen system. **Results:** Following oral administration, HEL-specific cellular immune responses were enhanced in HEL low-responder mice, and the concentrations of gamma interferon (IFN- γ), but not interleukin (IL)-4, increased significantly. In addition, the prescriptions decreased the level of HEL-specific antibodies of the immunoglobulin (Ig)G1 subtype, which is associated with helper T lymphocyte (Th2) cell stimulation. Moreover, the presence of the medicines in vitro significantly increased IFN- γ production from mouse splenocytes, and the magnitude of the increase was closely associated with glycoprotein concentrations. **Conclusions:** The Korean prescriptions enhanced anti-HEL-specific cellular immune responses by selectively priming specific subtypes of the helper T cell population. Consequently, they might be useful therapy for patients who need enhanced Th1, or to suppress Th2 immune responses.

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Keywords: Antibody; Antigen; Cytokine; Traditional Korean medicine; Immune modulation

Abbreviations: HEL, hen egg-white lysozyme; Ig, immunoglobulin; TdR, thymidine deoxyribose; IL, interleukin; IFN- γ , gamma interferon; Th, helper T lymphocyte; SA, Soamsan; BHT, Bo-yang-hwan-o-tang.

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1. Introduction

Traditional oriental medicines include mixtures of natural products such as herbs, animal derivatives, and minerals. Typical Korean traditional medicines consist of 5 to 15 components that are carefully mixed to minimize side effects and maximize medicinal effects [1,2]. Traditional medicines have long been used to

treat human diseases, especially diseases of the elderly. Recently, there has been increasing interest in the specific biological activity of traditional medicines, and numerous studies support their potential clinical benefit for diseases that are difficult to treat such as cancer [3–5]. Traditional medicines appear to exert their pharmacological actions through the synergistic effects of their components and via drug interactions. Therefore, they may modulate biological responses, including immune responses, rather than acting through the direct activity of the individual components [6,7].

Of the traditional medicines, Soamsan (SA) and Boyang-hwan-o-tang (BHT) have long been used to treat a variety of diseases and maintain good general health [8,9]. Both medicines are also used to treat cancers; they are thought to induce cancer remission and prevent chronic illnesses by modulating immune responses. In previous studies, we investigated whether traditional medicines could act as immune modulators. Using hen egg-white lysozyme (HEL) as a model antigen system, we showed that oral administration of the medicines potentially enhanced antigen-specific antibody as well as T cell-mediated immune responses [10,11]. In the present study, we prepared two mixed prescriptions composed mainly of components used in SA and/or BHT. Based on previous results and on the principles of traditional oriental medicine, we attempted to strengthen cell-mediated immune responses in mice, taking into account synergistic effects and interactions between the components. We then determined the effect of the prescriptions on HEL-specific immune response modulation, and demonstrated that the prescriptions effectively regulated antigen-specific cell-mediated immune responses. In addition, the immunological basis of the working mechanism is discussed.

2. Materials and methods

2.1. Chemicals, plastics, and mice

Unless otherwise specified, all chemicals and plastics used in this study were from Sigma (St. Louis, MO) and Falcon Labware (Becton-Dickinson, Franklin Lakes, NJ), respectively. Cytokine reagents were obtained from BD PharMingen (San Diego, CA). Four- to six-week old inbred C57BL/6 mice from Damul Science (Yusung, Korea) were housed five/

age following standard guidance of laboratory animals during the experimental periods.

2.2. Preparation of the new prescriptions

All the herbal materials to prepare the new prescriptions, named Soam I (SAI) and Soam II (SAII), were from the College of Oriental Medicine, Wonkwang University (Iksan, Korea). SAI and SAII were prepared by extracting the mixture of 15 and 12 medicinal herbs, respectively, which are the main components of SA and/or BHT, and the compositions of each prescription are shown in Table 1. The extraction of the herbal mixture was performed as described previously [10].

2.3. Application of the prescriptions and immunization of mice

Five mice/group were fed either SAI or SAII dissolved in PBS, in oral doses of 250 or 500 mg/kg body weight, similar doses as in clinical use considering the human body weights, every other day for 1 week. On

Table 1
Composition of SAI and SAII

Components		Grams	
SAI	SAII	SAI	SAII
<i>Angelicae gigantis</i> Radix	<i>Angelicae gigantis</i> Radix	30	30
<i>Astragalus mongholicus</i> Bunge	<i>Astragalus</i> <i>mongholicus</i> Bunge	30	60
<i>Atractylodes</i> <i>macrocephalae</i> Koidz	<i>Carthamus</i> <i>tinctorius</i> L.	20	10
<i>Corydalis</i> <i>turtschaninovii</i> Besser	<i>Corydalis</i> <i>turtschaninovii</i> Besser	15	20
Ginseng radix	Ginseng radix	30	60
<i>Helianthus annuus</i> L.	<i>Helodrilus foetidus</i>	30	20
<i>Laminaria japonica</i>	<i>Ligusticum</i>	12	30
Aresch <i>Ligusticum chuanxiong</i> Hort	<i>chuanxiong</i> Hort <i>Oldenlandia diffusa</i> Roxb	15	25
<i>Scutellaria barbata</i> D. Don	<i>Paeonia lactiflora</i> Pall	30	30
<i>Oldenlandia diffusa</i> Roxb	<i>Paeonia suffruticosa</i> Andr.	25	20
<i>Paeonia suffruticosa</i> Andr.	<i>Prunus persica</i> Batsch	15	10
<i>Rheum undulatum</i> L.	<i>Rheum undulatum</i> L.	25	20
<i>Sargassum stenophyllum</i>		12	
<i>Spargranii</i> Rhizoma		10	
<i>Tribuli fructus</i>		30	

day 7 of the feeding, mice were immunized subcutaneously with 50 μg of HEL emulsified in complete Freund's adjuvant. The mice were then fed again the same doses every third day until boost immunization was performed with the same amount of the antigen emulsified in incomplete Freund's adjuvant at 10 days after the initial immunization. In parallel, a control group of mice was treated similarly with the same volume of PBS instead of SAI and SAII. Sera were collected from the blood drawn on the fourth day after the boost immunization to evaluate the level of HEL-specific humoral immune responses.

To evaluate the level of HEL-specific lymphocyte proliferation and cytokine production, splenocytes were collected from mice 10 days after the initial immunization without a boost immunization. In this set of experiment, mice were fed either SAI or SAII every other day throughout the experimental period.

2.4. HEL-specific antibody responses

The level of HEL-specific immunoglobulin (Ig) in the mice sera was determined by ELISA as described previously [12]. Briefly, each well of the ELISA plate was coated with 1 μg of HEL and blocked with PBS containing 10% FBS (HyClone, Logan, UT) and 0.05% Tween-20. After washing the wells with PBS, 50 μl of the individual samples, serially diluted in 10% FBS in PBS, was added to each well and incubated for 2 h at 37 $^{\circ}\text{C}$. Goat anti-mouse Ig was used as a secondary antibody to determine the total amount of HEL-specific antibodies. Alternatively, rat antibodies specific to mouse Ig subclasses such as $\gamma 1$, $\gamma 2\text{a}$, $\gamma 2\text{b}$, and $\gamma 3$ were used to determine the amount of HEL-specific antibodies of IgG1, IgG2a, IgG2b, and IgG3 subtypes, respectively. Finally, substrate was added to develop the color, and the absorbance was measured at 405 nm using a SpectraCountTM (Packard Instrument, Downers Grove, IL) ELISA reader.

2.5. HEL-specific lymphocyte responses

Single cell suspensions (2×10^6 cells/ml) of splenocytes were prepared from either control mice or the mice fed SAI or SAII as described above and spread onto 96-well flat bottom plates (100 μl /well). The cells were stimulated with varied concentration of HEL (0.1–30 $\mu\text{mol/l}$) and labeled with 1 $\mu\text{Ci/ml}$ of [meth-

^3H] thymidine deoxyribose (TdR) (Amersham Pharmacia Biotech, Piscataway, NJ) for the last 12 h during a 72-h culture period. The cells were then harvested using a cell harvester (Inotech, Switzerland) and the incorporated tritium contents were determined using a liquid scintillation counter (Packard Instrument).

2.6. HEL-specific cytokine production

The amount of cytokines produced by HEL-stimulated lymphocytes was determined by ELISA, which was serviced by the Bank for Cytokine Research (Chonbuk National University, Chonju, Korea), as described previously [13]. Briefly, splenocytes prepared as described above were stimulated in 24-well flat bottom plates with varied concentration of HEL (0.1–30 $\mu\text{mol/l}$) for 72 h. Culture supernatants were

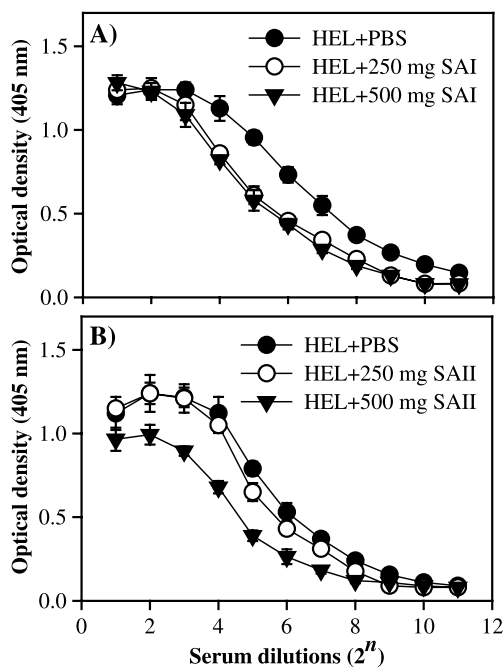


Fig. 1. Effect of the new prescriptions on anti-HEL IgG production by HEL low-responder C57BL/6 mice. The mice were fed SAI (A) and SAII (B) and then immunized with HEL. Data were obtained from ELISA performed with equal volumes of sera pooled from five mice/group taken 4 days after booster immunization. The same experiments were repeated three times and a representative result is shown. Data represent the level of anti-HEL antibodies and are expressed as the mean \pm S.E.

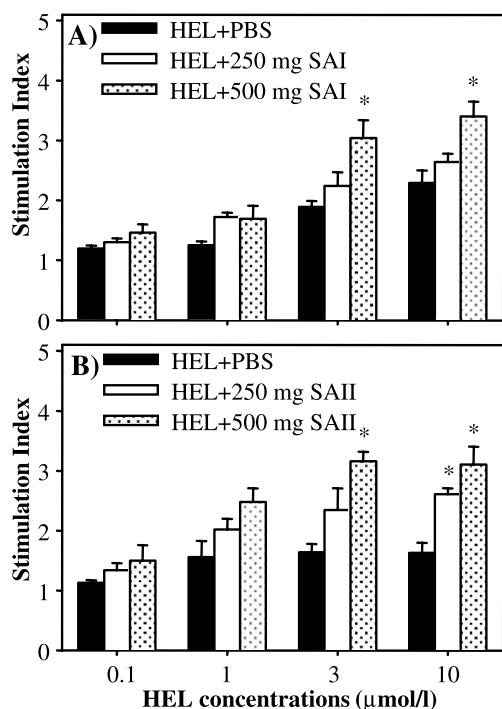


Fig. 2. Anti-HEL T cell proliferative responses of splenocytes prepared from C57BL/6 mice fed with new prescriptions. C57BL/6 mice were fed 250 or 500 mg/kg SAI (A) and SAII (B). Splenocytes from the mice were collected 10 days after a single immunization with HEL and incubated with the indicated concentrations of HEL for 72 h. Proliferative responses were quantified by measuring the [methyl- 3 H]TdR uptake. Each bar represents the stimulating indices (SI) \pm S.E. calculated by dividing the cpm of tested samples with that of controls from triplicate cultures. * P < 0.05 indicates a significant difference between the experimental and control values. The same experiments were repeated three times and a representative result is shown.

then collected and processed for ELISA using the IFN- γ - and interleukin (IL)-4-specific OptiEIA™ kit (BD PharMingen). Cytokine concentrations were calculated based on standard curves generated using known concentrations of recombinant cytokine proteins.

2.7. In vitro assays on proliferation, cytokine production, and cytotoxicity

To assay the effect of prescriptions on lymphocyte proliferation and cytokine production by immune cells in vitro, splenocytes (2×10^6 cells/ml) prepared from naive C57BL/6 mice were incubated in 0.5% FBS-supplemented RPMI-1640 medium with varied con-

centrations of either SAI or SAII (10–200 μ g/ml). Subsequently, 1 μ Ci/ml of [methyl- 3 H]TdR was added onto each well for the last 12 h during a 48-h culture period. In addition, culture supernatants from either SAI- or SAII-stimulated cells for 48 h were collected and then the amounts of cytokines representing the stimulation of helper T lymphocyte (Th)1-type (gamma interferon (IFN- γ) and IL-2) and Th2-type (IL-4) T cells were determined by ELISA. SAI- and SAII-mediated cytotoxicity was also measured by trypan blue exclusion assay [14].

2.8. Characterization of active components

In order to characterize the nature of stimulating activity of the prescriptions on splenocytes, crude

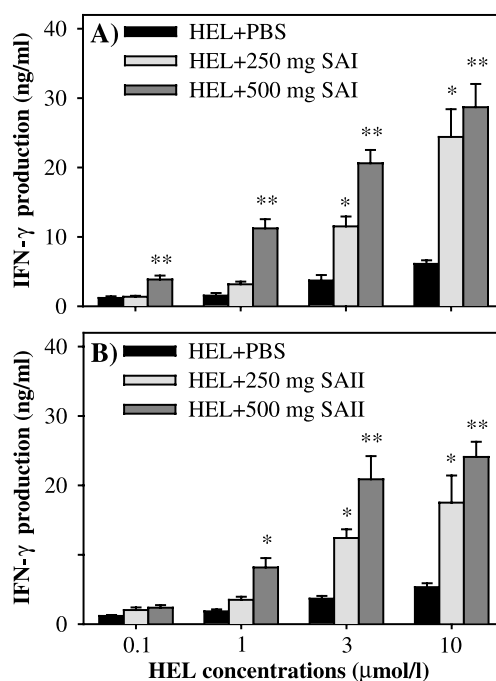


Fig. 3. IFN- γ production by antigen stimulation of splenocytes prepared from C57BL/6 mice fed either SAI (A) or SAII (B). All procedures for the in vivo treatment, including medicines and HEL immunization, and the in vitro stimulation by HEL were conducted as described in Fig. 2. Culture supernatants were collected 72 h after in vitro stimulation with HEL, and cytokine levels were measured by ELISA. The results are expressed as the mean \pm S.E. * P < 0.05 and ** P < 0.01 represent significant differences between the experimental and control values. The same experiments were repeated three times and a representative result is shown.

protein preparations were prepared from the original SAI and SAII extracts as described previously [10]. The crude protein preparations were initially analyzed through SDS-PAGE with 10–15% polyacrylamide gel. The gels were then stained either with Coomassie brilliant blue to detect total proteins or with Schiff reagent to detect glycoproteins [15].

In addition, crude protein preparations were treated with either pronase E or NaIO₄ to degrade either proteins [16] or carbohydrate residues [17], respectively. Briefly, crude proteins (5 mg) were incubated in 4 ml of 0.1 M Tris–HCl buffer (pH 8.0) containing 0.6 mg of pronase E and 50 mmol/l CaCl₂ for 72 h at 30 °C. The reaction mixtures were then heated at 100 °C for 10 min to inactivate pronase E and dialyzed against PBS before passing through the Sephadex G-150 column. Alternatively, the crude proteins (5 mg) were incubated with 100 µl of 0.1 mol/l NaIO₄ at 25 °C for 4 h followed by the addition of 250 µl of 20% ethylene glycol. Then, the samples were dialyzed and applied to the column as described above.

To determine whether the crude protein preparations contain lipopolysaccharide (LPS)-like compounds, the crude proteins (200 µg) were incubated

for 2 h at 37 °C in 500 µl of culture medium containing 500 units of polymyxin B (PMB) before adding to splenocytes culture and tested if the PMB treatment could inhibit the proliferation mediated by LPS-like compounds.

2.9. Statistical analyses

All the results were expressed as mean ± standard error (S.E.). A one-way ANOVA using SPSS v 10.0 software was used to make a statistical comparison among the groups and a value of $P < 0.05$ was considered significant.

3. Results

3.1. Oral administration of SAI and SAII negatively modulated anti-HEL humoral immune responses

HEL-specific IgG concentrations were lower in sera from mice fed SAI than in sera from control mice (Fig. 1A). When mice were fed with SAII, the HEL-specific IgG level also decreased, and the

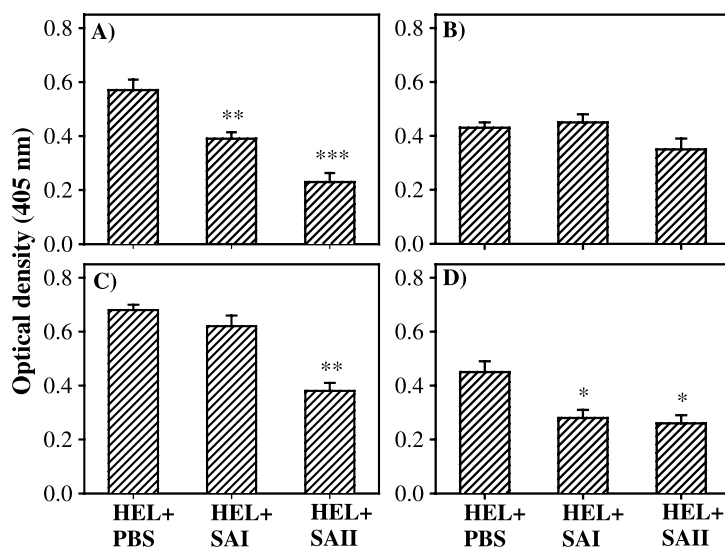


Fig. 4. The effect of either SAI or SAII administration on immunoglobulin subclass levels after HEL immunization. C57BL/6 mice were fed 500 mg/kg of either SAI or SAII and immunized with HEL, as described in the Materials and methods section. In this experiment, isotype-specific secondary antibodies were used to identify the immunoglobulin subclasses, including IgG1 (A), IgG2a (B), IgG2b (C), and IgG3 (D). The results represent values obtained with sera diluted 128-fold and are expressed as the mean ± S.E. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ indicate significant differences between the experimental and control values. The same experiments were repeated three times and a representative result is shown.

degree of reduction was greater in mice given 500 mg/kg SAI than in those given 250 mg/kg SAI (Fig. 1B). These results suggest that oral administration of either SAI or SII negatively modulated the induction of HEL-specific antibody immune responses.

3.2. Oral administration of either SAI or SII enhanced HEL-specific lymphocyte proliferative responses

When spleen cells were stimulated with HEL (0.1–10 $\mu\text{mol/l}$) in vitro, a greater proliferative response was seen in mice fed either SAI or SII than in mice fed with PBS alone (Fig. 2A and B). This indicates that oral administration of the new prescriptions enhances the induction of HEL-specific lymphocyte stimulation, thereby enhancing cell-mediated immune responses.

3.3. Oral administration of newly mixed prescriptions increased IFN- γ but not IL-4

After antigen stimulation, IFN- γ concentrations were higher in mice fed either SAI or SII than in control mice fed with PBS alone (Fig. 3A and B). When splenocytes prepared from mice fed 500 mg/kg of either SAI or SII were restimulated with 10 $\mu\text{mol/l}$ HEL, they produced 28.71 and 24.10 ng/ml IFN- γ , respectively. Splenocytes from control mice produced 5.32 ng/ml. However, IL-4 expression by HEL-stimulated splenocytes was not significantly different in treated and control mice, even at the highest HEL concentration tested (data not shown). These results suggest that oral administration of either SAI or SII preferentially stimulates Th1 cells or, alternatively, suppresses the selective priming of Th2 cells.

3.4. Level of HEL-specific antibody isotypes correlated with cytokine levels

Oral administration of either SAI or SII significantly decreased serum titers of HEL-specific IgG1, which is known to be closely related to IL-4 levels (Fig. 4). The decrease was greater in mice fed SII than those fed SAI (Fig. 4A). Unexpectedly, however, the level of HEL-specific IgG2a, which is known to

be closely related to IFN- γ levels, was not increased significantly (Fig. 4B). Interestingly, oral administration of the prescriptions decreased the concentrations of IgG2b and/or IgG3 subtypes, which are known to be associated with the direct stimulation of B lymphocytes (Fig. 4C and D). These results suggest that modulation of the HEL-specific immune response induced by prescriptions is controlled by the balance between Th1 and Th2 cell-mediated immune responses, either by selective Th1 cell priming or by suppressing Th2 cell stimulation.

3.5. Immune-stimulating activity of SAI and SII extracts and crude protein preparations in vitro

As shown in Table 2, the prescriptions significantly increased splenocyte tritium uptake. When 100 $\mu\text{g/ml}$ of either SAI or SII was added to splenocytes, tritium uptake increased 4.07- and 4.08-fold, respectively, compared to control cells (4722 ± 272 cpm). Furthermore, crude SAI and SII protein preparations induced significantly more splenocyte proliferation

Table 2
Effect of SAI and SII extracts and crude protein preparation on mouse splenocyte proliferation

Stimulants ($\mu\text{g/ml}$)	[methyl- ^3H]TdR uptake by splenocytes (cpm)	
	SAI	SII
Control	4722 \pm 272	
<i>Whole mixture</i>		
10	7458 \pm 710*	7049 \pm 264*
50	11,367 \pm 1100**	12,349 \pm 1495**
100	19,197 \pm 1260***	19,246 \pm 2306***
200	22,563 \pm 1991***	20,258 \pm 2707***
<i>Crude protein</i>		
10	25,240 \pm 1614***	20,665 \pm 1444***
50	31,471 \pm 1522***	24,084 \pm 2200***
100	32,940 \pm 2263***	28,273 \pm 1802***
200	30,860 \pm 2403***	29,278 \pm 1470***

C57BL/6 spleen cells (2×10^6 cells/ml) were incubated with either the original SAI and SII preparations or crude proteins for 48 h and labeled with [methyl- ^3H]TdR for the last 12 h. The cells were then harvested, and the tritium uptake was measured. The results are expressed as the mean tritium uptake in cpm \pm S.E. in triplicate cultures.

* $P < 0.05$ was significantly different from the control value.

** $P < 0.01$ was significantly different from the control value.

*** $P < 0.001$ was significantly different from the control value.

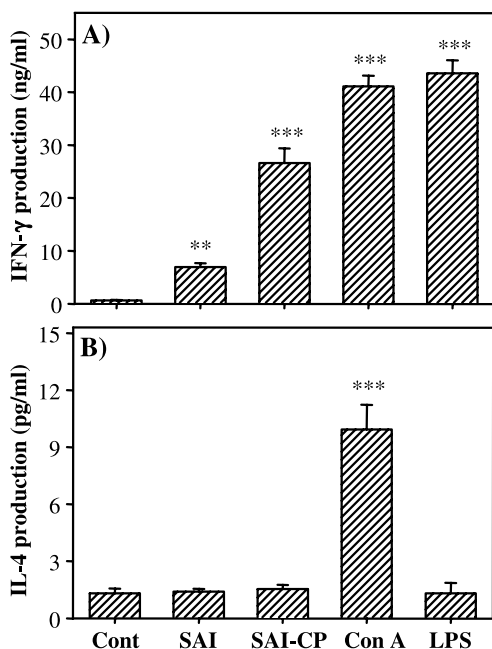


Fig. 5. The effect of SAI on cytokine production in splenocytes prepared from naive mice. Splenocytes were cultured and exposed to 10 $\mu\text{g/ml}$ of the original SAI and its crude protein preparation (SAI-CP) for 48 h. The levels of (A) IFN- γ and (B) IL-4 were analyzed by ELISA. Con A and LPS were used as positive controls, and each bar represents the mean \pm S.E. ** $P < 0.01$ and *** $P < 0.001$ represent significant differences between the experimental and control values.

than the original SAI and SAII preparations at the same concentrations. Additionally, trypan blue exclusion experiments showed that neither SAI nor SAII had any significant cytotoxic effect on cultured splenocytes, even at the highest concentration tested (data not shown).

As shown in Fig. 5A, splenocytes treated with SAI produced significantly more IFN- γ than controls. When 10 $\mu\text{g/ml}$ SAI, or a crude SAI protein preparation, was added to cells, IFN- γ levels were 10.78- and 41.61-fold higher, respectively, than untreated cells (0.64 ng/ml). However, SAI treatment did not affect IL-4 levels (Fig. 5B). SAII treatment induced a similar pattern of cytokine production (data not shown). These results clearly support the assumption that the balance between Th1 and Th2 stimulation controls the induction of antigen-specific immune responses following oral administration of SAI and SAII.

3.6. Glycoproteins were responsible for the splenocyte-stimulating activity of the new prescriptions

Crude protein samples enhanced both lymphocyte proliferation and cytokine production, and this activity was significantly higher than the activity induced by SAI and SAII extracts at the same concentration. This finding suggested that the crude protein preparations contained the active compounds responsible for the induction of antigen-specific immune responses. To characterize the crude proteins, we separated the samples using SDS-PAGE, and found a band of around 100 kDa that clearly stained with Schiff reagent (data not shown). To establish whether the splenocyte-stimulating activity of the crude SAI and SAII protein samples was due to glycoproteins, we treated the samples with either pronase E or NaIO₄, and then tested their ability to stimulate proliferation and cytokine production. As shown in Fig. 6, treating the samples with either pronase E or NaIO₄ markedly, but not completely, reduced the stimulatory activity of the crude proteins. Treatment also reduced IFN- γ production in vitro (data not shown). Interestingly, polymyxin B, an inhibitor of the LPS response, did not reduce the stimulating activity of the samples, whereas it clearly abolished LPS-induced B cell proliferation (Fig. 7). These results clearly support the hypothesis that glycoproteins in the crude protein

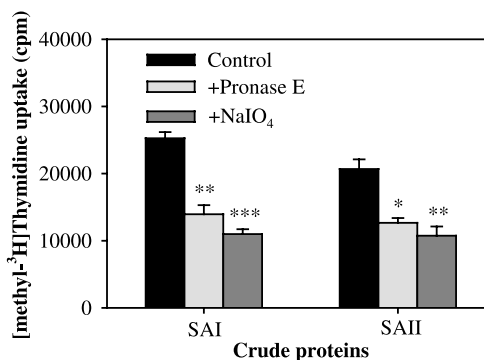


Fig. 6. Inhibitory effect of pronase E and NaIO₄ on the splenocyte-stimulating activity of crude proteins. Crude proteins from either SAI or SAII were incubated in the absence or presence of pronase E and NaIO₄, and their proliferation induction activity was estimated by a tritium incorporation assay. 10 $\mu\text{g/ml}$ of each sample was added to splenocyte cultures. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ indicate significant differences between the experimental and control values.

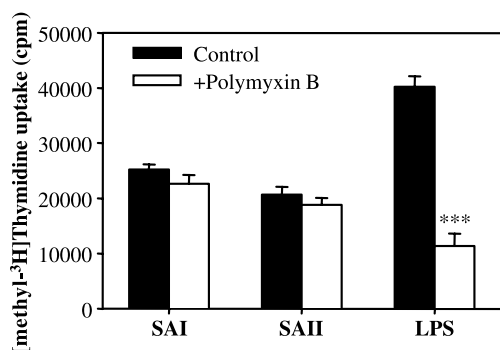


Fig. 7. The effect of polymyxin B on the splenocyte-stimulating activity of crude proteins. Crude proteins (200 μ g) from either SAI or SAII, and LPS (50 μ g) were incubated for 2 h at 37 $^{\circ}$ C in 500 μ l of culture medium containing 500 units of polymyxin B (PMB). 10 μ g/ml of each sample was added to splenocyte cultures. *** P < 0.001 indicates a significant difference between the experimental and control values.

preparation were mainly responsible for splenocyte stimulation, and that this activity was not due to contamination by LPS-like molecules.

4. Discussion

We examined the effect of two new prescriptions of traditional Korean medicines on anti-HEL antibody production and the lymphocyte proliferative response (Figs. 1 and 2). Oral administration to mice significantly enhanced splenocyte tritium uptake, but not antigen-specific serum IgG levels. This suggests that treatment with either SAI or SAII enhances the induction of cell-mediated immune responses rather than humoral immune responses to antigen.

In an attempt to understand the nature of the modulated HEL-specific immune responses induced by oral SAI and SAII treatment, we determined IFN- γ and IL-4 levels after splenocyte stimulation because the balance between Th1 and Th2 cell stimulation plays an important role in modulating the immune response induction [18,19]. As shown in Fig. 3, the cytokine ELISA revealed that IFN- γ levels were significantly higher in culture supernatants of antigen-stimulated splenocytes from prescription-fed mice than in supernatants from control mice. In contrast, IL-4 levels were not affected (data not shown). Moreover, HEL-specific antibody titers with IgG1 subtype, known to be asso-

ciated with Th2 cell stimulation, were clearly decreased in mice fed with the new prescriptions (Fig. 4). We therefore postulated that oral administration of SAI and SAII selectively stimulated Th1 cells rather than Th2 cells by increased IFN- γ vs. IL-4, and was likely to play an important role in modulating anti-HEL-specific immune responses in this study [20–22]. This assumption is further supported by results from in vitro splenocyte stimulation assays in which production of IFN- γ , but not IL-4, was greatly increased by adding the prescriptions (Fig. 5).

There has been argument for the use of oriental medicines regarding quality control since the contents of plant components such as carbohydrates, proteins, moisture, and minerals could be changed depending on the seasons and grown areas. Generally, however, it has been noted that main ingredients and quality of herbal plants in traditional Korean medicines have been regularly checked through random sampling. Moreover, we obtained the components used to prepare the prescriptions from other sources and repeated the exactly same experiments to assure the quality of the mixed prescriptions used in this study. Consequently, we could exclude the possible argument for quality control of the mixed prescriptions of the traditional medicines used in this study.

One of the problematic aspects of traditional medicines is poor characterization of the active ingredients responsible for the action of medicines. Although we still believe that the best way of application for the oriental medicines is to use as a mixture rather than as an active ingredient since the prescriptions were prepared based on careful consideration of the principles of oriental medicines and the synergistic effects among ingredients used to prepare the prescription, we tried to characterize the active ingredient responsible for the immune modulation activity as shown in this study. Glycoproteins in both SAI and SAII were thought to be the active components in lymphocyte stimulation and cytokine production (Table 2 and Fig. 5), and it has been reported that the stimulatory effects of many oriental medicinal herbs on lymphocyte proliferation and T cell cytokine production are associated with glycoproteins [16,23,24]. This finding was confirmed by the inhibition of stimulatory activity by treatment with pronase E and NaIO₄ (Fig. 6), which degrade proteins and carbohydrates, respectively and is in agreement with results from previous studies [10,11].

Hence, we believe that glycoproteins in mixed prescriptions might have an important role in modulating antigen-specific cell-mediated immune responses, although the extent of glycoprotein involvement should be characterized further.

Immunologically, the Th1/Th2 cytokines interact reciprocally to maintain a balanced immune network [21,25,26]. 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 this study, we examined the modulating activity of mixed prescriptions of traditional medicines, SAI and SAI1, on antigen-specific immune responses in vivo and in vitro. Our results suggest that both medicines potentially enhance Th1-mediated or, alternatively, suppress Th2-mediated immune responses. Therefore, we speculate that these new prescriptions could be used for immunotherapy in patients with skewed immune responses such as perennial allergic rhinitis [27]. To confirm the speculation, we are considering the application of new prescriptions on animal models of diseases caused by depressed Th1 or abnormally elevated Th2 immune responses. In addition, we are concentrating our efforts on characterizing the active ingredients, including glycoproteins, contained in these prescriptions.

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