

# Persimmon leaf extract and astragaloside inhibit development of dermatitis and IgE elevation in NC/Nga mice

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**Background:** We previously found that persimmon leaf extract contains antiallergic substances that inhibit histamine release by human basophilic cell line KU812 in response to cross-linkage of FcεRI.

**Objectives:** The purpose of this study was to identify substances in the persimmon leaf extract that are responsible for the effect and to examine their in vivo effects on the allergic mouse model.

**Methods:** HPLC analysis of persimmon leaf extract was done to measure its content. Inhibitory activity of persimmon leaf extract or its major constituent of flavonoids (astragaloside) on the histamine release by KU812 cells was examined. To investigate the effects of these substances in vivo, models of passive cutaneous anaphylaxis and atopic dermatitis mice (NC/Nga) were used.

**Results:** Persimmon leaf extract or astragaloside inhibited histamine release from KU812 in response to cross-linkage of FcεRI. Oral intake of both substances dose dependently inhibited passive cutaneous reactions. Moreover, oral administration of these substances to NC/Nga atopic dermatitis-model mice led to a striking suppression of the development of dermatitis, scratching behavior, and serum IgE elevation. Histologic analyses revealed that infiltration of inflammatory cells, especially degranulated mast cells, thickening of the epidermis, and prominent hyperkeratosis, were significantly reduced. Immunologic studies showed that the capacity of spleen T cells to produce both IL-4 and IL-13, but not IFN-γ, was downregulated by means of oral intake of these substances.

**Conclusion:** This study demonstrates a novel activity of astragaloside and the dramatic effect of persimmon leaf extract and astragaloside on atopic dermatitis-model mice. (*J Allergy Clin Immunol* 2000;106:159-66.)

**Key words:** Persimmon leaf extract, astragaloside, histamine, passive cutaneous reaction, NC/Nga mouse, atopic dermatitis, skin, hypersensitivity, IgE

Atopic dermatitis is a common disease and has been recently increasing in industrialized countries.<sup>1</sup> Recent immunologic analyses of the pathogenesis of this disease have revealed that activated mast cells and excess of differentiated T<sub>H</sub>2 cells through chemical mediators and cytokines might play major roles in the development of dermatitis and IgE synthesis.<sup>2,3</sup> Although it has been accepted that topical steroid therapy is crucial for the management of the disease,<sup>4,5</sup> many patients are still dissatisfied with its effectiveness and are worried about chronic use of this drug.

The persimmon *Diospyros kaki thunberg* (Ebenaceae) grows in China, Korea, and Japan. It was found that tea brewed from the leaves of this persimmon tree had a beneficial effect on hemostasis, diuresis, constipation, and hypotension. Flavonol kaempferol, one of the flavonoids, was found in a hot water extract from persimmon leaves.<sup>6</sup> Kaempferol has been reported to inhibit antigen- or ionophore A23187-induced histamine secretion from rat mast cells,<sup>7,8</sup> DNP-BSA antigen-induced hexosaminidase secretion from RBL-2H3 cells,<sup>9</sup> and phorbol myristate acetate-induced O<sub>2</sub> production from human neutrophils.<sup>10</sup> Kaempferol-3-glucoside (astragaloside) from persimmon leaves was found to possess hypotensive action through its suppression of angiotensin-converting enzyme activity.<sup>11,12</sup> Kaempferol and astragaloside from the white petals of *Impatiens balsamina* L were shown to have an antipruritic effect on dextran T40-evoked scratching behavior in mice.<sup>13</sup> However, so far there is little in the literature on the antiallergic effect of astragaloside. In a previous study we showed that persimmon leaf extract inhibited histamine release from human basophilic cell line KU812 and the 24-hour homologous passive cutaneous anaphylaxis (PCA) reaction in mice.<sup>14</sup> In the present study we examined the inhibitory effects of persimmon leaf extract and of astragaloside, which is considered to be the primary ingredient for the action of persimmon leaf, on a human basophilic cell line KU812 and on NC/Nga atopic dermatitis model mice.

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**Abbreviations used**

FcεRI: High-affinity IgE receptor  
PCA: Passive cutaneous anaphylaxis

**METHODS****Culture medium**

RPMI-1640 supplemented with 10% FCS (Hyclone Laboratories, Logan, Utah), L-glutamine (2 mmol/L), 2-mercaptoethanol (0.05 mmol/L), penicillin (100 U/mL), and streptomycin (100 µg/mL) was used as the culture medium.

**Chemicals**

Persimmon leaf from Shi-chuan, China, was purchased at a retail shop in Japan. Kaempferol-3-glucoside was purchased from Extrasynthese (Genay, France), RPMI-1640 was purchased from Gibco BRL (New York, NY), epinephrine was purchased from Daiichi Pharmaceutical Co Ltd (Tokyo, Japan), histamine EIA kit was purchased from Immunotech A Beckman Coulter Co (Marseille, France), and CRA-1 (anti-FcεRIα mAb)<sup>15</sup> was purchased from Kyokuto Pharmaceutical Industry Co Ltd (Tokyo, Japan). The MF diet and α-corn starch were obtained from Oriental Bioservice Co Ltd (Kyoto, Japan), and A23187 was purchased from Sigma Japan (Tokyo).

**Preparation of persimmon leaf extract**

The persimmon leaf was steeped in boiling distilled water for 5 minutes and filtered through a nylon mesh (pore size, 40 µm). After centrifugation at 5000 rpm for 20 minutes at 4°C, the supernatant of the persimmon leaf extract was freeze-dried. The contaminated endotoxin level in persimmon leaf extract and astragaline was less than 1 ng/mL.

**HPLC quantitative analysis of astragaline in persimmon leaf extract**

Astragaline was quantified by means of RP-HPLC in a separative column of YMC-pack ODS-A A-312 (6 × 150 mm; particle size, 5 µm) with water/acetonitrile/2-propanol (180:38:2) as the mobile phase, a flow rate of 1.0 mL/min at room temperature, and UV detection at 350 nm. Astragaline quantification was carried out in duplicate and determined by comparison with a standard astragaline absorption curve.

**Histamine release assay**

KU812 cells (human basophilic leukemia cells)<sup>16</sup> were grown in RPMI-1640 supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO<sub>2</sub> in air at 37°C. KU812 cells were suspended in a histamine release buffer (30 mmol/L TRIS-HCl [pH 7.6], 120 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L CaCl<sub>2</sub>, 1 mmol/L MgCl<sub>2</sub>, and 0.03% BSA). The first incubation of 100 µL of KU812 cells (10<sup>5</sup> cells/mL) was performed with 100 µL of persimmon leaf extract (0.02%, 0.01%) and astragaline (0.0001%, 0.00005%) in an ice bath for 30 minutes. After centrifugation at 3000 rpm for 5 minutes at 4°C, 200 µL of histamine release buffer was added to the cells removed from the supernatant, which was then incubated with 15 µg/mL CRA-1 at 37°C for 30 minutes. After centrifugation at 3000 rpm for 5 minutes at 4°C, the histamine content of the supernatant and cell lysate was measured by means of ELISA assay. Positive and negative controls were used to indicate histamine content obtained with or without CRA-1.

**PCA reaction**

PCA reaction was evaluated according to the previous method.<sup>17</sup> Briefly, 6-week-old male ddY mice were obtained from Japan SLC

(Shizuoka, Japan) and housed at 23°C ± 3°C in 55% ± 15% humidity with 12 hour-12 hour light-dark cycle (light on 7 AM-7 PM). After preliminary breeding for 3 days, the mice were divided into 6 groups of 3 each. Twenty microliters of anti-DNP IgE antibody (10 µg/mL; Yamasa Shouyu Co Ltd, Chiba, Japan) was intracutaneously injected into the ears of ddY mice. Twenty-four hours after the sensitization, persimmon leaf extract (100 or 250 mg/kg) and astragaline (0.6 or 1.5 mg/kg) were orally administered by using a metal stomach tube. To the control group and the no treatment group, distilled water was orally administered in the same manner. After 1 hour, mice were challenged with 100 µL of DNP-BSA (1 mg/mL) intravenously. Fifteen minutes thereafter, the thickness of the ear was measured with a thickness gauge 3 times. The ear swelling was calculated according to the following equation:

Ear swelling (mm) = Ear thickness after 15 minutes intravenously – Ear thickness after 0 minutes intravenously

**Continuous feeding of persimmon leaf extract or astragaline in NC/Nga mice and evaluation of the development of dermatitis**

Four-week-old NC/Nga mice (conventional grade) were obtained from Japan SLC (Shizuoka, Japan) and housed at 23°C ± 3°C in 55% ± 15% humidity with 12 hour-12 hour light-dark cycle (light on 7 AM-7 PM). After preliminary breeding for 1 week, the mice were divided into 3 groups of 5 each and provided with one of the following diets ad libitum: control diet (MF diet, Oriental Inc), persimmon leaf extract diet (MF diet containing 0.125% persimmon leaf extract, 250 mg/kg), or astragaline diet (control diet plus 0.00075% astragaline, 1.5 mg/kg) during the 8-week experiment. The severity of dermatitis was assessed once a week with the following scoring system. Symptoms were evaluated by skin dryness, eruption, and wound on the 3 parts of the body (ie, ear, face, and head and back). Each symptom was graded from 0 to 3 (no symptoms, 0; mild, 1; moderate, 2; and severe, 3). Blood was taken once every 2 weeks from the eyes, and the serum IgE level was measured by means of an enzyme immunoassay with mAbs (Yamasa Shouyu Co Ltd, Chiba, Japan). The serum levels of immunoglobulins, including IgM, IgG1, IgG2a, IgG2b, and IgG3 at the age of 13 weeks, were also measured by using the Mab-Based Mouse Ig Isotyping kit (PharMingen, San Diego, Calif). Serum was obtained by centrifugation at 3000 rpm for 5 minutes at 4°C and stored at –80°C until use. The frequency of scratching behavior of 13-week-old NC/Nga mice, such as scratching of the nose, ears, and dorsal skin with the hind paws, was measured during a 20-minute period. Licking of the belly and dorsal skin during grooming was disregarded. Each occurrence of scratching of the head, neck, dorsal skin, ears, eyes, and nose was scored to obtain the maximum score.

**Histologic study**

Whole ears of the mice at 13 weeks of age were fixed in a formalin solution. A block of the skin was removed and embedded in paraffin, cut in 10-µm sections, and stained with hematoxylin and eosin or toluidine blue.

**Preparation of spleen cells**

Cell suspensions were prepared from spleens. Red blood cells were lysed with the ammonium-chloride potassium carbonate buffer, and nucleated cells were washed 3 times and resuspended in the culture medium.

**FACS analysis**

Cells were stained with FITC anti-CD3, phycoerythrin anti-CD4, or FITC-anti-B220 antibody (PharMingen Inc, San Diego, Calif) for 1 hour at 4°C. After washing, the number of positive cells

was determined by cytometric analysis on a FACScalibur (Becton Dickinson and Co, Mountain View, Calif) by using PI gating to limit the analysis to living cells.

### Lymphokine assays

Spleen cells ( $2 \times 10^6$ /mL) were cultured with soluble anti-CD3 (1  $\mu$ g/mL) plus anti-CD28 (1  $\mu$ g/mL) antibodies (Pharmingen) in a 24-well Costar plate (Corning Inc, Cambridge, Mass). Supernatants were collected at 48 hours, and IL-4, IL-13, and IFN- $\gamma$  concentrations were measured with a 2-site ELISA (Pharmingen).

### T-cell proliferation

Spleen cells ( $10^6$  cells/mL) were incubated in 96-well, flat-bottom, microtiter plates (Costar, Cambridge, Mass) with or without anti-CD3 (1  $\mu$ g/mL) plus anti-CD28 (1  $\mu$ g/mL) antibodies for 3 days in triplicate. For the last 8 hours of culture, 0.5  $\mu$ Ci per well of tritiated ( $^3$ H) thymidine was added. The cells were harvested onto filters, and  $^3$ H-thymidine incorporation was determined by using liquid scintillation spectroscopy.

### Statistics

The Student *t* test was used for the statistical analysis.

## RESULTS

Freeze-dried extract was obtained from persimmon leaf (0.004 g from 1 mL of persimmon leaf and 0.06 g from 1 g of persimmon leaf). Astragalin (Fig 1) content was determined by means of HPLC at 0.59 g of astragalin per 100 g of persimmon leaf extract (Fig 1).

### Both persimmon leaf extract and astragalin inhibit histamine release from human basophilic cell line KU812 in response to Fc $\epsilon$ RI cross-linkage

To examine whether this material could inhibit histamine release from basophilic cells, human cell line KU812 was used. The cross-linkage of Fc $\epsilon$ RI by the anti-Fc $\epsilon$ RI $\alpha$  chain antibody CRA-1 in KU812 cells induced histamine release as previously demonstrated.<sup>14</sup> One representative result of 4 experiments shown in Fig 2 demonstrated that spontaneous secretion of histamine content in the supernatants was  $2.75 \pm 0.10$  nmol/L and that stimulation generated  $11.08 \pm 1.13$  nmol/L of histamine in the supernatants. Preincubation of KU812 with epinephrine suppressed its secretion by 40% to 60% in a dose-dependent fashion in every experiment (data not shown). The presence of persimmon leaf extract and astragalin consistently inhibited histamine release, and in this experiment the following inhibition of the release was observed:  $38\% \pm 12\%$  ( $P < .05$ ) with 0.01% and  $33\% \pm 9\%$  ( $P < .05$ ) with 0.005% persimmon leaf extract;  $40\% \pm 2\%$  ( $P < .01$ ) with 0.00005% (1.11  $\mu$ mol/L) and  $25\% \pm 14\%$  with 0.000025% astragalin.

### Effect of oral administration of persimmon leaf extract or astragalin on the passive cutaneous anaphylaxis in mice

To investigate whether persimmon leaf extract or its major flavonoid astragalin might show antiallergic

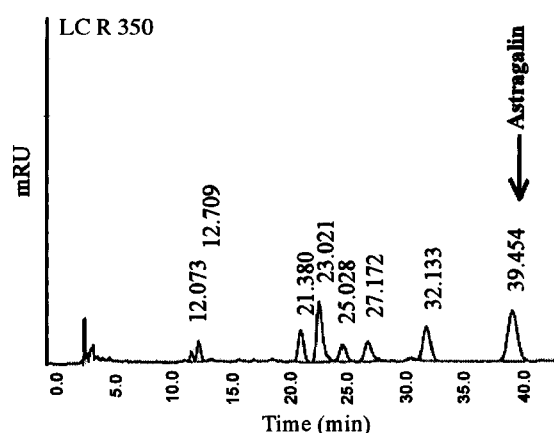
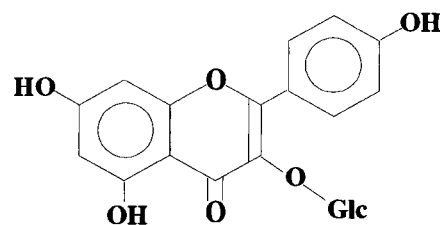


**FIG 1.** Chemical structure of astragalin and chromatogram of persimmon leaf extract monitored by UV detector. HPLC conditions: column, YMC-pack ODS-A A-312; mobile phase, water/acetonitrile/2-propanol (180:38:2); flow rate, 1.0 mL/min; detection wavelength, 350 nm.

effects *in vivo*, a model of PCA was used. ddY mice were injected subcutaneously into ears by 20  $\mu$ L of anti-DNP IgE antibody (10  $\mu$ g/mL), and 24 hours later they were orally administered with persimmon leaf extract or astragalin. Thickness of the ear 15 minutes after antigen challenge with DNP-BSA was measured. Fig 3 shows that oral intake of persimmon leaf extract or astragalin inhibits the PCA reaction in a dose-dependent fashion.

### Oral administration of persimmon leaf extract or astragalin inhibits serum IgE elevation and development of dermatitis in NC/Nga atopic dermatitis model mice

Subsequently, to examine whether persimmon leaf extract or astragalin might be effective for allergic diseases *in vivo*, NC/Nga mice were used. NC/Nga mice have been recently recognized as atopic dermatitis-model mice.<sup>18,19</sup> This mouse spontaneously develops severe eczema with aging under nonspecific pathogen free (SPF) circumstances. In addition, the histology of its dermatitis mimics human atopic dermatitis, and the

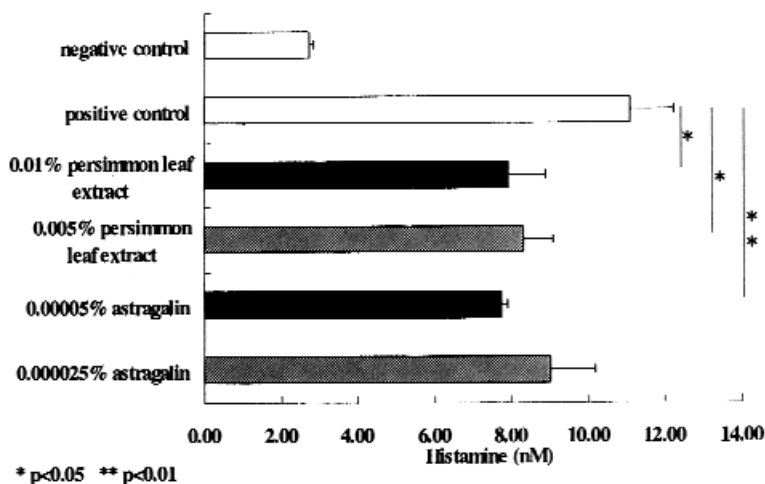


**FIG 2.** Inhibitory effect of persimmon leaf extract and astragalalin on histamine release from KU812 cells stimulated with anti-Fc $\epsilon$  receptor mAb (CRA-1). Cells ( $10^4$  cells) were at first incubated with persimmon leaf extract (final concentrations, 0.01% and 0.005%) and astragalalin (final concentrations, 0.00005% and 0.000025%) on ice for 30 minutes and incubated with 15  $\mu$ g/mL CRA-1 at 37°C for 30 minutes. Histamine content of the supernatant was measured by means of ELISA. Positive and negative controls indicate histamine content with or without CRA-1. Values represent the mean  $\pm$  SD of 3 determinations. \* $P < .05$  and \*\* $P < .01$ ; significantly different from positive control.

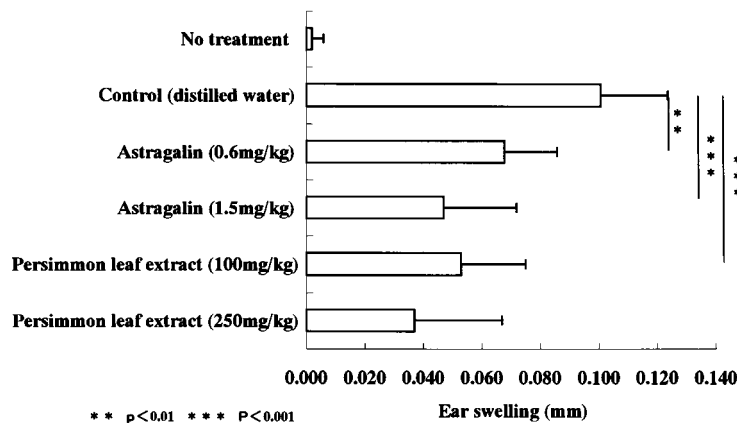


**FIG 3.** Inhibition of PCA reaction by means of persimmon leaf extract or astragalalin in ddY mice. ddY mice were injected with anti-DNP IgE antibody. Persimmon leaf extract or astragalalin was orally administered into mice, and 1 hour later, DNA-BSA was challenged. Fifteen minutes later, the thickness of both ears was measured. Values represent the mean  $\pm$  SD of 6 determinations from 3 mice. \*\* $P < .01$  and \*\*\* $P < .001$ ; significantly different from positive control.

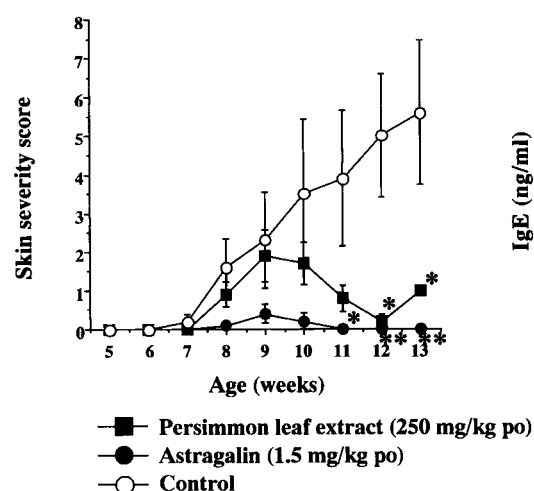
serum IgE level is significantly elevated after the onset of dermatitis. Five mice in each group were orally given persimmon leaf extract (250 mg/kg), astragalalin (1.5 mg/kg), or a control diet. Development of dermatitis was observed in the control mice: mild eczema of the head and neck at 10 weeks of age; mild to moderate eczema and hemorrhage of the head and neck, dorsal skin, and ears at 11 weeks; and clear alopecia of the armpit and breast at 13 weeks. However, oral intake of persimmon leaf extract or astragalalin remarkably inhibited the appearance of these skin symptoms. Representative skin is shown in Fig 4. The skin conditions were evaluated weekly by artificial skin severity score, and the results

are shown in Fig 5. There was a striking difference in skin severity score between controls and persimmon leaf extract- or astragalalin-treated mice. Oral administration of astragalalin almost completely suppressed the development of dermatitis and also inhibited scratching behavior (data not shown). The histologic study revealed that in control mice at 13 weeks of age, thickening of the epidermis, prominent hyperkeratosis, and infiltration of inflammatory cells were observed (Fig 6). Morphologic study or staining showed that these infiltrating cells in the dermis were degranulated mast cells with a substantial number of eosinophils and lymphocytes. These changes were more severe in the outside than the inside



**FIG 4.** Clinical features of NC/Nga skin. NC/Nga mice were provided with one of the following diets ad libitum: **A**, control diet (MF diet containing 0.125% of  $\alpha$ -corn starch,  $n = 5$ ); **B**, persimmon leaf extract diet (MF diet containing 0.125% of persimmon leaf extract at 250 mg/kg,  $n = 5$ ); or **C**, astragalol diet (control diet plus astragalol at 1.5 mg/kg,  $n = 5$ ) during the 8-week experiment. The photograph was taken at 13 weeks.

## 1. Skin score



## 2. Serum IgE

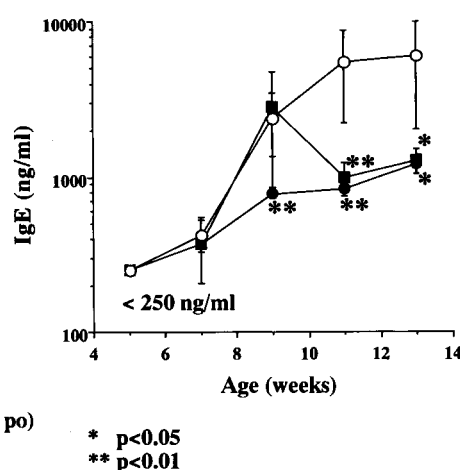


**FIG 5.** Effect of persimmon leaf extract or astragalol on clinical scores of skin symptoms and serum IgE level. Clinical scores and serum IgE level were measured. Values represent the mean  $\pm$  SD. \* $P < .05$  and \*\* $P < .01$ ; significantly different from control diet.

of the ear. Oral intake of persimmon leaf extract or astragalol also inhibited thickening of the epidermis, hyperkeratosis, and the infiltration of inflammatory cells in the dermis, as shown in Fig 6. Thus these results indicate that oral intake of persimmon leaf extract or its major flavonoid astragalol inhibits the development of dermatitis in NC/Nga mice. Furthermore, the serum level of IgE was also decreased by persimmon leaf extract or astragalol (Fig 5). Serum level of IgE was measurable after 7 weeks of age and was gradually increased with aging. At any time except for 9 weeks of age in persimmon leaf extract mice, there was a significant difference of IgE between control and other groups of mice. In the 13-week-old NC/Nga mice, serum level of IgE was decreased 5-fold by persimmon leaf extract or astragalol. However, as shown in Table I, other isotypes of immunoglobulins, such as IgM, IgG1, IgG2a, IgG2b, and IgG3, in the sera were not decreased by oral intake of astragalol.

## Oral administration of persimmon leaf extract or astragalol inhibits IL-4 and IL-13 synthesis by spleen T cells

Next, to identify the mechanisms through which IgE elevation is suppressed by oral intake of astragalol, immunologic analyses were carried out when the mice were 13 weeks old. FACS examination of two mice from each group showed that the total number of spleen cells was similar and that the level of CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, or B220<sup>+</sup> cells showed no significant difference between control and astragalol-treated mice (Table II). Then the capacity of spleen T cells to produce IL-4, IL-13, and IFN- $\gamma$  was measured after anti-CD3 plus anti-CD28 antibody stimulation. Fig 7 shows that the synthesis of T<sub>H</sub>2 cell-derived cytokines IL-4 and IL-13 was significantly diminished ( $P < .05$ ) by oral intake of astragalol but that there was little change in IFN- $\gamma$  synthesis. Finally, the proliferative response of spleen T cells with anti-CD3

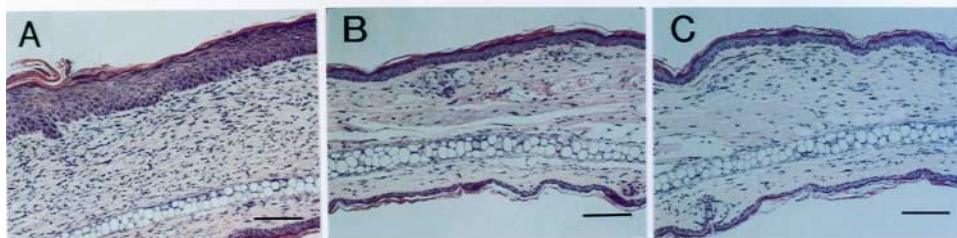


**FIG 6.** Histologic features of skin lesions. Hematoxylin and eosin-stained sections of the ear from control NC/Nga mice (**A**), persimmon leaf extract mice (**B**), or astragaline mice (**C**).

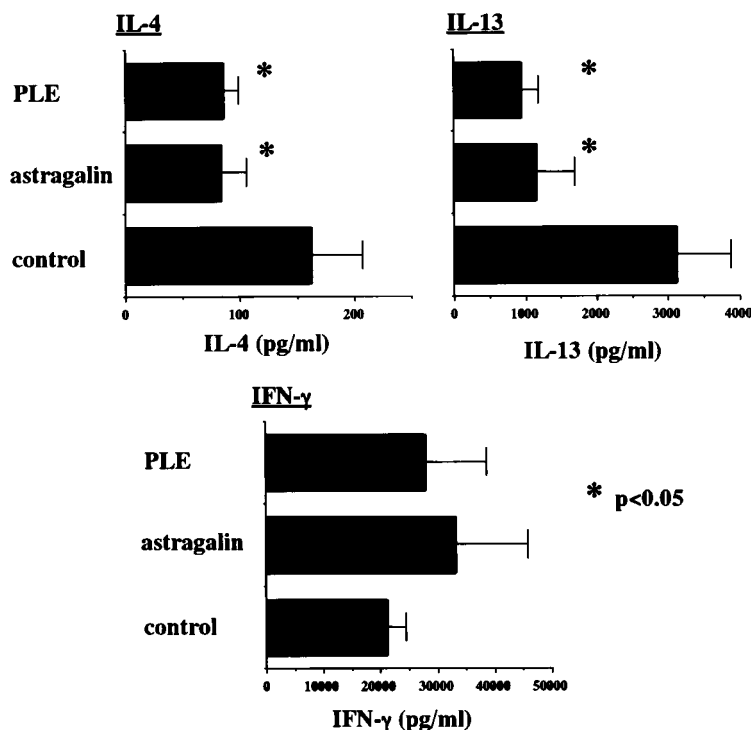


**FIG 7.** IL-4 and IL-13 synthesis by spleen cells from astragaline or persimmon leaf extract (PLE) mice is suppressed. Spleen cells from mice at 13 weeks of age were stimulated with anti-CD3 plus anti-CD28 antibodies for 2 days. IL-4, IL-13, or IFN- $\gamma$  in the culture supernatants was measured by ELISA. Values represent the mean  $\pm$  SD. \* $P$  < .05.

plus anti-CD28 antibodies was studied. Spleen cells were incubated with or without both antibodies for 3 days, and then  $^3\text{H}$ -thymidine incorporation was measured. No stimulation caused  $437 \pm 37$  cpm from control mice and  $111 \pm 19$  cpm from astragaline-treated mice. The stimulation of spleen cells with anti-CD3 plus anti-CD28 antibodies resulted in  $29,173 \pm 4492$  cpm from control mice and  $32,408 \pm 2727$  cpm from astragaline-treated mice. Thus this suppressive effect of astragaline on IL-4 and IL-13 synthesis was not due to a general inhibition of T cell-proliferative response.

## DISCUSSION

We previously reported that persimmon leaf extract contains antiallergic substances that inhibit histamine

release by KU812 cells in response to cross-linkage of Fc $\epsilon$ RI and that oral administration of persimmon leaf extract suppressed PCA.<sup>14</sup> In this study we demonstrated that the major constituent in the persimmon leaf extract, which is responsible for the inhibition of histamine release, might be astragaline and that oral administration of not only persimmon leaf extract but also astragaline inhibited passive cutaneous anaphylaxis reaction in vivo. In addition, both substances prevented the development of dermatitis and IgE elevation in NC/Nga atopic dermatitis model mice.

It has been reported that botanical materials include several constituents with antiallergic and anti-inflammatory actions.<sup>20</sup> Typical examples of such plants are *Perilla frutescens*, *Camellia sinensis*, *Rubus suavissimus*, *Artemisia princeps*, and *Aloe arborescens*. Antiallergic

**TABLE I.** Serum immunoglobulin concentrations in 13-week-old mice

|                    | IgM       | IgG1        | IgG2a       | IgG2b       | IgG3      | IgE         |
|--------------------|-----------|-------------|-------------|-------------|-----------|-------------|
| Control (μg/mL)    | 842 ± 532 | 3500 ± 1785 | 2534 ± 1194 | 8636 ± 3752 | 34 ± 14.8 | 6.0 ± 3.9   |
| Astragalín (μg/mL) | 750 ± 253 | 3476 ± 2117 | 1684 ± 564  | 6028 ± 1730 | 30 ± 2.6  | 1.22 ± 0.3* |

Serum concentrations of Igs from 5 mice were measured by using ELISA. Values are shown as means ± SE. A paired Student *t* test is used to assess the level of significance of the difference (*P* < .05).

**TABLE II.** Cell population in the spleens of 13-week-old mice

|                | CD3 <sup>+</sup> | CD4 <sup>+</sup> | CD8 <sup>+</sup> | B220 <sup>+</sup> | non-T, non-B |
|----------------|------------------|------------------|------------------|-------------------|--------------|
| Control (%)    | 20.4 ± 0.7       | 13.7 ± 0.9       | 8.2 ± 0.3        | 64.5 ± 0.3        | 13.6 ± 3.5   |
| Astragalín (%) | 27.6 ± 5.8       | 19.5 ± 4.6       | 8.8 ± 1.0        | 63.1 ± 2.3        | 9.3 ± 2.3    |

Whole spleen cells were stained with appropriate FITC- or phycoerythrin-labeled mAb. Positive cells were determined by using FACS analysis. Data is shown as the mean ± SD from two spleens. There is no significant difference between each group.

substances, such as flavonoids, catechins, and caffeines, contained in these plants have been shown to be responsible for these actions. Catechins are known to suppress histamine release of rat mast cells and to inhibit TNF- $\alpha$  production by LPS-stimulated mouse macrophages.<sup>21-23</sup> (–)-Epigallocatechin, (–)-epicatechin gallate, and (–)-epigallocatechin gallate are the main constituents in oolong tea, green tea, and black tea extracts. These extracts are also known to include caffeines with antiallergic activities.<sup>24</sup> However, in persimmon leaf extracts these 3 major catechins ([–]-epigallocatechin, [–]-epicatechin gallate, and [–]-epigallocatechin gallate) and caffeines could not be detected. Persimmon leaves are reported to include 0.15% astragalín and 0.04% isoquercetin from MeOH extract<sup>12</sup> and 0.09% astragalín, 0.004% isoquercetin, 0.008% quercetin-3-(2 *o*-galloyl)-glucoside, and 0.014% kaempferol-3-(2 *o*-galloyl)-glucoside from Me<sub>2</sub>CO-H<sub>2</sub>O (7:3) as flavonoids.<sup>11</sup> The present persimmon leaf extract used in our study contained 0.59% astragalín. Because pure astragalín also suppressed histamine release from KU812 cells at a dose equivalent to that in the persimmon leaf extract, it is conceivable that the main constituent that is responsible for this inhibition is astragalín in the persimmon leaf extract. Previous reports indicate that fisetin, kaempferol, quercetin, and myricetin have an inhibitory effect on histamine release from rat peritoneal mast cells.<sup>8</sup> The effect of astragalín on the inhibition of histamine release in human blood cells was found to be twice as strong as that of kaempferol (data not shown). In addition, Cheong et al<sup>9</sup> examined the structure activity relationship of flavonoids for antiallergic actions through analyses of their inhibitory activity on hexosaminidase release from RBL-2H3 rat mast cells. Apigenin, luteolin, 3,6-dihydroxyflavone, fisetin, kaempferol, quercetin, and myricetin were found to be the flavonoids, which strongly inhibit hexosaminidase release, with an inhibitory concentration of 50% at a level of around 1.8 to 7.5 μmol/L. A comparison of the effects of concentrations of such flavonoids on inhibition with references in the literature suggests that astragalín is one of the strongest inhibitors among flavonoids.

To determine whether persimmon leaf extract and astragalín have antiallergic effects *in vivo*, ddY and NC/Nga mice were used. Oral administration of each material strongly inhibited the PCA reaction. In NC/Nga mice spontaneous development of dermatitis and IgE elevation under non-SPF circumstances are observed.<sup>18,19</sup> Furthermore, histologic analyses have shown that their skin lesions mimic those of human atopic dermatitis. The administration of persimmon leaf extract by mouth led to a striking inhibition of skin symptoms and IgE elevation. Because astragalín showed a similar suppressive effect on dermatitis and on IgE elevation *in vivo*, these results support the finding that a major antiallergic substance in persimmon leaf extract is astragalín, although it cannot be completely denied that other components in persimmon leaf extract may additively contribute to the effect. Kaempferol and astragalín from the white petals of *I. balsamina* L have been shown to have an antipruritic effect on dextran T40-evoked scratching behavior in mice.<sup>13</sup> As for IgE suppression by botanical materials *in vivo*, only *P. frutescens* extract has been demonstrated to inhibit anti-DNP IgE production in mice immunized with DNP-ovalbumin in alum adjuvant.<sup>25</sup>

The clinical efficacy of persimmon leaf extract and astragalín on the inhibition of dermatitis may have been mediated through their inhibitory effect on histamine release by FcεRI<sup>+</sup> cells because the histologic study revealed that there were few degranulated mast cells in the dermis of mice who were given astragalín. Furthermore, these materials had an inhibitory effect on IgE synthesis *in vivo*. It has been shown that IgE synthesis by B cells is primarily regulated by cytokines; T<sub>H</sub>2-derived cytokines, including IL-4 and IL-13; IgE class-switching factors, upregulated IgE synthesis; and T<sub>H</sub>1-derived cytokines, especially IFN- $\gamma$ , are strong inhibitors of IgE synthesis.<sup>26-28</sup> In addition to T<sub>H</sub>2 cells, basophils, mast cells, and NK1.1T cells have been shown to be IL-4 producers,<sup>29,30</sup> and in fact peripheral blood basophils and tissue localized mast cells were found to produce IL-4.<sup>31-33</sup> We found that oral intake of astragalín or persimmon leaf extract suppressed IL-4 and IL-13 production, but not IFN- $\gamma$  synthesis, by spleen T cells in response to anti-CD3

plus anti-CD28 antibodies. These changes in the cytokine profile might contribute to IgE suppression. Whether astragalin directly may affect the differentiation of T cells into  $T_H2$  cells remains to be determined. We would like to point out here that preliminary experiments revealed that astragalin at the dose of 10 to 30  $\mu\text{mol/L}$  did not inhibit IgE synthesis by LPS plus IL-4-stimulated mouse B cells nor did it suppress IL-4 synthesis by spleen non-B, non-T cells in response to plate-bound IgE plus IL-3. However, persimmon leaf extract and a hydroxylated flavonoid of astragalin, kaempferol, which is possibly converted from astragalin in vivo, showed an inhibitory activity of IL-4 synthesis by non-B, non-T cells.

The presence of astragalin in plants is ubiquitous, and intake of this natural constituent may be effective for allergic disease. Persimmon leaf extract contains more astragalin than is found in other materials. We hope that these observations may contribute to the clinical application of these materials as alternative medicines for allergic diseases.

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