

Control of IgE and selective T_H1 and T_H2 cytokines by PG102 isolated from *Actinidia arguta*

Eun-Jin Park, PhD,^a Bongcheol Kim, PhD,^b Haekwan Eo, MS,^b Kyungcheol Park, MS,^b Yeonran Kim, PhD,^b Hwa Jun Lee, MD,^c Miwon Son, PhD,^d Yoon-Seok Chang, MD,^e Sang-Heon Cho, MD,^e Sunyoung Kim, DPhil,^a and Mirim Jin, PhD^b *Seoul, Suwon, and Youngin, Korea*

Background: Various allergic responses are thought to result from the unbalanced development of T_H1 and T_H2 pathways and, subsequently, the overproduction of IgE. Therefore the modulation of T_H1 and T_H2 responses is a rational strategy for the treatment of allergic diseases.

Objective: The present study was performed to investigate the immune-modulating activities of PG102 preparations from *Actinidia arguta* in ovalbumin-sensitized murine models.

Methods: Two preparations from *A. arguta*, PG102T and PG102E, were chosen for animal experimentation on the basis of their ability to regulate the production of IgE in U266B1 cells. The changes in splenic levels of cytokines and plasma levels of immunoglobulin isotypes were examined. The effects of PG102 on subcellular composition (CD4⁺IL-4⁺ or CD19⁺IgE⁺ cells), IgE production in B cells, and selective transcription factors were analyzed.

Results: Oral administration of PG102T and PG102E significantly decreased the level of selective T_H2 cytokines, whereas it increased the level of T_H1 cytokines. The differential effects of PG102T and PG102E on T_H1 and T_H2 cytokines were accompanied by a decrease in the plasma levels of IgE and

IgG1 and by an increase in the plasma level of IgG2a. The percentages of both IL-4-producing T cells and IgE-producing B cells were decreased. The concentration of IgE produced within B cells also appeared to be reduced. Finally, PG102T and PG102E downregulated the level of GATA-binding protein 3, while inducing that of T-box transcription factor and nuclear factor of activated T cells c2.

Conclusion: PG102T and PG102E have great potential as orally active immune modulators for the therapy of various allergic diseases. (J Allergy Clin Immunol 2005;116:1151-7.)

Key words: *Actinidia arguta*, PG102T, PG102E, T_H1/T_H2 cytokines, IgE, GATA-3, T-bet, NFATc2

Allergic responses involve the degranulation of IgE-dependent mast cells and the accumulation of eosinophils at the sites of inflammation, which are considered to result from the unbalanced overactivation of T_H2 cells and, consequently, the T_H2-mediated overproduction of IgE.¹⁻³ The representative cytokines of T_H2 cells, such as IL-4, IL-5, IL-10, and IL-13, are known to play important roles in these reactions. Furthermore, T_H1-mediated cytokines, such as IFN- γ and IL-12, were reported to negatively regulate the T_H2 pathways.^{1,3-5} Various cellular transcription factors, such as GATA-binding protein 3 (GATA-3) and T-box transcription factor (T-bet), control the differentiation of T_H1 and T_H2 cells and the production of cytokines in these cells.⁶⁻⁹

Because a bulk of evidence suggested that the T_H1 and T_H2 types of reactions are reciprocally regulated *in vivo*, the modulation of T_H1 and T_H2 pathways has been thought to be one of the more rational strategies for developing immune modulation-based antiallergy therapeutics.¹⁰ For example, recombinant cytokines, such as IL-12 and IFN- γ , or cytokine receptor antagonists to IL-4 and IL-5 have been tested for their abilities to control the balance between T_H1 and T_H2 responses.^{11,12} However, these reagents are not orally active and often cause undesirable side effects. Thus there is a need for development of an orally active and safe immune modulator for the therapy of allergic diseases. In an effort to find such immune modulators, we have been screening for natural reagents from plants and fruits on the basis of the rationale described in the Online Repository in the online version of this article at www.jacionline.org. Here, we report that 2 preparations from *A. arguta*, PG102T and PG102E, have

From ^aSchool of Biological Sciences and Institute of Molecular Biology and Genetics, Seoul National University; ^bPanGenomics Co Ltd, Biotechnology Incubating Center, Seoul; ^cKyungHee Vincent Oriental Hospital, Suwon; ^dDong-A Co Ltd, R&D Center, Youngin; and ^eInstitute of Allergy and Clinical Immunology, Seoul National University Medical Research Center. Supported by a grant from the Plant Diversity Research Center (PF0320303-00) of 21C Frontier R&D Programs, Ministry of Science and Technology (Korea).

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Reprint requests: Sunyoung Kim, DPhil, and Mirim Jin, PhD, Institute for Molecular Biology and Genetics 105-306, Seoul National University, San 56-1, Shillim-dong, Kwanak-gu, Seoul 151-742, Korea. E-mail: sunyoung@plaza.snu.ac.kr.

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Abbreviations used

DEX:	Dexamethasone
DW:	Distilled water
GATA-3:	GATA-binding protein 3
IC ₅₀ :	Inhibitory concentration of 50%
NFATc2:	Nuclear factor of activated T cells c2
OVA:	Ovalbumin
PG102:	A general term for various preparations from <i>Actinidia arguta</i>
PG102B:	<i>n</i> -Butanol-soluble fraction from PG102T
PG102C:	Chloroform-soluble fraction from PG102T
PG102E:	Ethylacetate-soluble fraction from PG102T
PG102T:	Total water-soluble extract from <i>Actinidia arguta</i>
PG102W:	Water-soluble fraction from PG102T
T-bet:	T-box transcription factor

inhibitory activities with regard to the production of IgE, as well as the ability to regulate selective T_H1 and T_H2 cytokines, probably by regulating the cellular transcription factors GATA-3, T-bet, and nuclear factor of activated T cells c2 (NFATc2). Our data indicate the great potential of PG102T and PG102E as natural immune modulators of the T_H1 and T_H2 pathways and ultimately as antiallergic reagents.

METHODS

Mice

Female BALB/c mice (6 weeks old) were obtained from Daehan Biolink, Co Ltd (Chungbuk, Korea), kept in an air-conditioned room, and acclimated for at least 1 week. All experimental procedures were performed in accordance with the institutional animal care and use guidelines of the Animal Experimental Center at Seoul National University.

Preparation of various extracts from *A arguta*

The hardy kiwifruits used in this study were purchased from a farm specializing in the cultivation of this fruit (Hurst's Berry Farm, Sheridan, Ore), and their identity was kindly confirmed by Dr Ella I. Kolbasina (The Moscow Branch of Vavilov Plant Cultivation Research Institute). Total water-soluble extract (PG102T) of the dried fruit and its various fractions were prepared as described by Kang et al.¹³ (For detailed methods concerning the extraction process, see the Online Repository in the online version of this article at www.jacionline.org.) PG102T or its fractions were dissolved in distilled water (DW) or 50% ethanol at a concentration of 100 mg/mL, respectively, and stored at -80°C until use. The PG102 preparations contained undetectable levels of endotoxin, as determined by using the Limulus amoebocyte lysate assay (Sigma, St Louis, Mo).

Bioassay in U266B1 cells and ovalbumin-stimulated splenocytes

The IgE-inhibitory effects of various PG102 preparations in LPS-stimulated human B lymphoblastoma cells (U266B1; ATCC, Manassas, Va) were measured, as described by Kim et al.¹⁴ (For a detailed description of this method, see the Online Repository in the online version of this article at www.jacionline.org.) The level of

human IgE in supernatants was detected by using human IgE ELISA (AlerChek, Portland, Me). Splenocytes from ovalbumin (OVA)-sensitized mice were prepared and cultured as described by Kato et al.¹⁰ and Tomkinson et al.¹² to determine the specific activity of PG102T and PG102E (see the Online Repository in the online version of this article at www.jacionline.org). After 3 days of culture, the supernatants were collected to measure the level of IL-4 by means of ELISA (Endogen, Cambridge, Mass).

Measurement of immunoglobulin levels in plasma and cytokine production by splenocytes from OVA-sensitized mice

Mice were individually immunized with OVA (20 μg) emulsified in aluminum hydroxide (2.25 mg) on days 0 and 14, respectively. OVA-sensitized mice ($n = 10$ per group) were orally treated with PG102T (15 mg/kg/d), PG102E (1.5 mg/kg/d), dexamethasone (DEX; 0.5 mg/kg/d), or DW (100 μL per mouse per day) once a day from day 14 to day 24 to assess the *in vivo* effects of PG102 on OVA-induced allergic responses. Naive mice were orally treated with DW. The concentrations of PG102T and PG102E used in this experiment are the minimal dosages having maximal activity in the preliminary dose-response experiment. On day 21, blood was collected from the retro-orbital plexus, and isolated plasma samples were kept at -80°C until use. The plasma level of total IgE was measured by using a mouse IgE detection kit (Shibayagi, Gunma, Japan). The levels of total IgG subtypes and OVA-specific immunoglobulin isotypes were determined by using the sandwich ELISA method, as described by Hirano et al.¹⁵

For the measurement of cytokine production, splenocytes were prepared from animals on day 24, resuspended in RPMI-1640 containing 10% FBS, seeded (5×10^6 cells/mL per well), and incubated in the presence or absence of OVA at 100 $\mu\text{g}/\text{mL}$ for 3 days. The levels of IL-4, IL-5, IL-10, IL-12, IL-13, and IFN- γ in supernatants were detected by using ELISA kits (Endogen and R&D Systems, Minneapolis, Minn).

Immunostaining and immunoblotting analyses

Splenocytes were stained with antibodies specific to mouse CD4, CD19, IL-4, IFN- γ , or IgE (all from PharMingen, San Diego, Calif) and then analyzed with a FACSsort analyzer and Cell Quest software (Becton Dickinson, San Jose, Calif), as described by Kyoko et al.¹⁶ (see the Online Repository in the online version of this article at www.jacionline.org). Confocal microscopic analyses were performed with the MRC-1024 Laser Scanning Confocal Image System (Bio-Rad Laboratories Inc, Hercules, Calif), as described by Semper et al.¹⁷ (see the Online Repository in the online version of this article at www.jacionline.org). Western blotting was performed with protein samples prepared from splenocytes (10^7 cells per group) and antibodies specific to mouse GATA-3, T-bet, NFATc2 (Santa Cruz Biotechnology, Santa Cruz, Calif), or β -actin (Sigma).

RNA preparation and quantitative real-time PCR

Total RNA was isolated from splenocytes cultured with OVA for 2 days by using TRIzol Reagent (GIBCO BRL, Carlsbad, Calif). Isolated total RNA was used in RT-PCR by using the AMV RT System (Roche, Mannheim, Germany), followed by quantitative real-time PCR with the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, Calif). Forward and reverse primer sets for mouse genes were designed with Primer Express Software (Applied Biosystems), and the nucleotide sequences are as

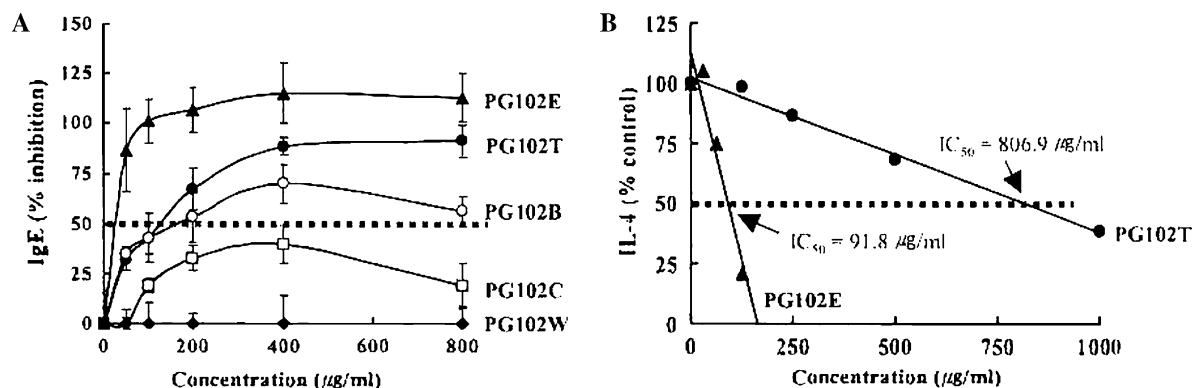


FIG 1. Inhibitory activity of various preparations from *A arguta* on the production of IgE in U266B1 cells (A) and IL-4 in OVA-stimulated splenocytes (B). Results were calculated as the percentage of IgE level produced from LPS-stimulated U266B1 cells and IL-4 level produced from OVA-stimulated splenocytes, respectively, from 3 independent experiments.

follows: GATA-3, forward 5'-CCTCGGCCATTCGTACATG-3' and reverse 5'-CGTAGTAGGACGGGACGTGG-3'; T-bet, forward 5'-TGTG-GATGTGGTC TTGGTGG-3' and reverse 5'-ATAAGC-GGTTCCCTGGCAT-3'; NFATc2, forward 5'-GCACATAAGG-CCATCAGCTCA-3' and reverse 5'-TCGCCAGAGAGACTGGC-AA-3'; reduced glyceraldehyde-phosphate dehydrogenase, forward 5'-TGCACTGGCAAAGTGGAGATT-3' and reverse 5'-TTGA-ATTTGCCGTGAGTGGGA-3'. The differences in mRNA levels of cellular genes between each group of mice were calculated from conversion of the threshold cycle (ΔC_t) value.

Statistics

Data were expressed as means $\pm$ SEM, and differences between mean values were analyzed by using the unpaired Student *t* test. *P* values of less than .05 or .01, which were calculated as 1-tailed *P* values, were considered statistically significant.

RESULTS

Effects of various preparations from *A arguta* in LPS-stimulated U266B1 cells

We initially prepared a total water-soluble extract (PG102T) from *A arguta* and subsequently extracted it with chloroform (PG102C), ethylacetate (PG102E), *n*-butanol (PG102B), and water (PG102W) to obtain 4 fractions soluble to a respective solvent. The effects of PG102T and the 4 fractions on IgE production were tested by using the human U266B1 cell line (Fig 1, A). Four of the preparations (all but PG102W) were found to inhibit the production of IgE from LPS-stimulated U266B1 cells. PG102E contained the highest inhibitory activity, displaying an inhibitory concentration of 50% (IC_{50}) at 25 μ g/mL. PG102T was next, with its IC_{50} value being 126 μ g/mL. In all concentrations tested in this assay, neither PG102T nor PG102E showed any cytotoxic effects on U266B1 cells (data not shown). These results indicated that PG102T and PG102E might contain the compound or compounds that directly inhibit the overproduction of LPS-stimulated IgE. On the basis of these data, we chose to use 2 preparations, PG102T and PG102E, for further *in vivo* studies.

Determination of specific activity of PG102T and PG102E

The complexity of traditional herbal medicines, which contain many constituents, makes the standardization of herb-derived products very difficult. To quantitatively perform further experimentation, we developed a reliable bioassay on the basis of the inhibitory activity of PG102 on the production of IL-4 in OVA-stimulated splenocytes. IL-4 was chosen as a biomarker for the quality control of PG102 because it plays important roles in both the production of IgE and the differentiation of T_H2 cells.¹⁻⁴ When cells were stimulated with OVA, the level of IL-4 was induced from an undetectable level to almost a hundreds picogram scale. The production of IL-4 was inhibited by PG102T or PG102E in a dose-dependent manner (Fig 1, B). In all concentrations tested in this assay, neither PG102T nor PG102E demonstrated any cytotoxic effects (data not shown). The IC_{50} value of PG102T was 806.9 μ g/mL, and that of PG102E was 91.8 μ g/mL. The IC_{50} value of each preparation was defined as 1 activity unit, and total units were obtained. When the specific activity of PG102T and PG102E was calculated by using total units and yield, PG102T and PG102E contained the specific activity of 1.2 units/mg and 10.9 units/mg, respectively. This method was used for the quality control of experimental samples from *A arguta*.

Effects of PG102T and PG102E on production of T_H1 and T_H2 cytokines by splenocytes from OVA-sensitized mice

The effects of PG102T and PG102E on the production of various cytokines involved in the modulation of T_H1 and T_H2 pathways were tested by using the OVA-sensitized murine model to confirm the above *in vitro* data in the animal model. OVA-sensitized mice were orally administered PG102T (15 mg [18 units]/kg/d) or PG102E (1.5 mg [16.4 units]/kg/d) and, as a control, DEX (0.5 mg/kg/d) or DW on a daily basis for 11 days. Naive

TABLE I. *In vivo* effects of PG102 on T_H1 and T_H2 cytokine production by splenocytes

Mouse	IL-12 (pg/mL)	IFN- γ (pg/mL)	IL-4 (pg/mL)	IL-5 (pg/mL)	IL-10 (pg/mL)	IL-13 (pg/mL)
Naïve	1353 $\pm$ 201 (394)	ND	ND	ND	77 $\pm$ 54 (2)	23 $\pm$ 17 (2)
OVA sensitized						
DW	343 $\pm$ 33 (100)	1023 $\pm$ 19 (100)	460 $\pm$ 42 (100)	1345 $\pm$ 68 (100)	3254 $\pm$ 298 (100)	1564 $\pm$ 132 (100)
PG102T	584 $\pm$ 136 (170)*	1970 $\pm$ 142 (193) $\dagger$	258 $\pm$ 31 (56)*	913 $\pm$ 79 (68)*	1812 $\pm$ 296 (56) $\dagger$	1220 $\pm$ 96 (78)
PG102E	889 $\pm$ 68 (259) $\dagger$	1778 $\pm$ 237 (174)*	164 $\pm$ 4 (36)*	412 $\pm$ 45 (31) $\dagger$	1591 $\pm$ 114 (49) $\dagger$	976 $\pm$ 304 (62)
DEX	227 $\pm$ 44 (66)*	224 $\pm$ 118 (22) $\dagger$	28 $\pm$ 21 (6)*	160 $\pm$ 159 (12)*	392 $\pm$ 157 (12) $\dagger$	912 $\pm$ 371 (58)

All splenocytes from OVA-sensitized mice were stimulated again with OVA. Values are expressed as the mean $\pm$ SEM for 10 animals. Values in parentheses are the relative percentage of when the percentage of the control (DW-treated mice) is 100.

ND, Not detectable.

* $P < .05$ and $\dagger P < .01$ versus DW-treated mice (Student *t* test).

TABLE II. Effects of PG102 on plasma levels of total immunoglobulin isotypes

Mouse	Total Ig			
	IgE (ng/mL)	IgG1 (μ g/mL)	IgG2a (μ g/mL)	IgG2b (μ g/mL)
Naïve	139 $\pm$ 23 (5)	450 $\pm$ 81 (26)	46 $\pm$ 1 (159)	229 $\pm$ 52 (85)
OVA sensitized				
DW	2783 $\pm$ 485 (100)	1700 $\pm$ 21 (100)	29 $\pm$ 3 (100)	270 $\pm$ 36 (100)
PG102T	1669 $\pm$ 319 (60)*	1009 $\pm$ 443 (59)*	67 $\pm$ 4 (231)*	325 $\pm$ 17 (120)
PG102E	1271 $\pm$ 247 (46)*	1446 $\pm$ 288 (85)	44 $\pm$ 4 (152)*	220 $\pm$ 65 (81)
DEX	1190 $\pm$ 317 (43)*	1614 $\pm$ 36 (95)	41 $\pm$ 7 (141)*	313 $\pm$ 82 (116)

Values are shown as means $\pm$ SEM for 10 animals.

* $P < .05$ versus DW-treated mice (Student *t* test).

TABLE III. Effects of PG102 on plasma levels of OVA-specific immunoglobulin isotypes

Mouse	OVA-specific Ig (% control of OD)			
	IgE	IgG1	IgG2a	IgG2b
Naïve	13 $\pm$ 1	5 $\pm$ 1	17 $\pm$ 1	51 $\pm$ 13
OVA sensitized				
DW	100	100	100	100
PG102T	29 $\pm$ 7 $\dagger$	76 $\pm$ 7*	217 $\pm$ 54*	104 $\pm$ 12
PG102E	27 $\pm$ 8 $\dagger$	69 $\pm$ 9*	304 $\pm$ 53 $\dagger$	84 $\pm$ 9
DEX	27 $\pm$ 8 $\dagger$	79 $\pm$ 11*	89 $\pm$ 25	84 $\pm$ 9

Values are shown as means $\pm$ SEM for 10 animals. Because OVA-specific antibodies were not available, the relative level of antibodies was calculated as the percentage of control OD.

* $P < .05$ and $\dagger P < .01$ versus DW-treated mice (Student *t* test).

mice were orally fed with DW. On day 24, mice were killed, isolated splenocytes from each group of mice were incubated in the presence or absence of OVA for 3 days, and cultured supernatants were collected to measure the levels of cytokines (Table I). Compared with naïve splenocytes, the level of IL-12 was decreased in DW-treated mice. However, oral administration of PG102T and PG102E increased its level by 1.7- and 2.6-fold, respectively. Unlike IL-12, the level of IFN- γ was enhanced by OVA stimulation, and PG102T or PG102E further increased the IFN- γ level. Contrary to PG102T and PG102E, DEX significantly suppressed the levels of both IL-12 and IFN- γ .

The level of all T_H2 cytokines tested in this study was highly increased in OVA-stimulated splenocytes. However, PG102T administration suppressed OVA-mediated overproduction of IL-4, IL-5, and IL-10 by 44%, 32%, and 44%, respectively. PG102E further inhibited the levels

of these 3 cytokines. DEX also lowered the concentration of all 3 cytokines. IL-13 was decreased by either PG102 or DEX but not in a statistically significant manner. Taken together, these results indicated that both PG102T and PG102E might control the production of selective T_H1 and T_H2 cytokines. Unlike DEX, which suppresses virtually all cytokines in a nondiscriminatory manner, PG102 appears to have distinctive biologic activities that can differentially modulate the production of T_H1 and T_H2 cytokines.

Effects of PG102T and PG102E on plasma levels of immunoglobulin isotypes

The above results suggested that PG102T and PG102E might downregulate T_H2-mediated overproduction of IgE *in vivo*. Therefore it was tested whether PG102T or PG102E in OVA-sensitized mice could control the plasma levels of IgE and other immunoglobulins. On day 21, naïve mice produced about 140 ng/mL total IgE, but

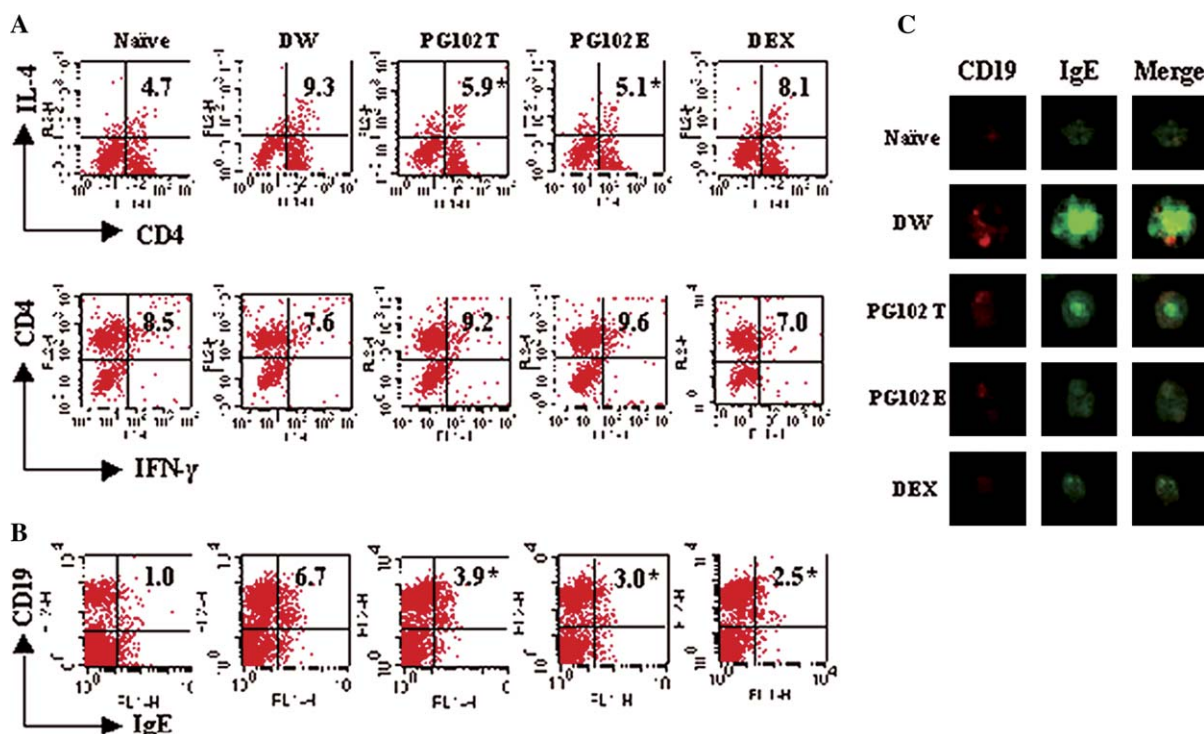


FIG 2. Effects of PG102T and PG102E on the number of IL-4- or IFN- γ -producing T cells (**A**) and IgE-producing B cells (**B**) and IgE biosynthesis within B cells (**C**). All splenocytes isolated from OVA-sensitized mice were stimulated again with OVA during the culture. Data are the mean of percentages of each population from 3 independent experiments. * $P < .05$ vs DW-treated mice.

sensitization with OVA increased this level by approximately 20-fold. When animals were treated with PG102T and PG102E, the plasma level of total IgE was decreased by about 2-fold. The capability of PG102T or PG102E to downregulate the level of total IgE was comparable with that of DEX. When the level of various IgG subtypes was measured, both PG102T and PG102E decreased the level of T_H2-mediated IgG1, whereas the level of T_H1-mediated IgG2a was highly increased in a statistically significant manner. The level of IgG2b was not significantly affected in all situations (Table II).

A virtually identical result was obtained when the levels of OVA-specific IgE and IgG subtypes were determined (Table III). Administration of PG102T or PG102E decreased the level of OVA-specific IgE and IgG1 while increasing that of OVA-specific IgG2a by more than 2-fold. These results, together with data on various T_H1 and T_H2 cytokines, indicated that PG102T and PG102E contain the activity that might skew the T_H1 and T_H2 balance, eventually resulting in an increase in the level of IgG2a and a decrease in that of IgE and IgG1.

Effects of PG102T and PG102E on T- and B-cell populations in splenocytes

To understand the cellular basis of the PG102T and PG102E activities described above, we tested how PG102 affected T- and B-cell populations present in splenocytes. Splenocytes from OVA-sensitized and naive mice were

grown in the presence or absence of OVA for 2 days, followed by FACS and confocal microscopic analyses. As shown in Fig 2, A, OVA stimulation increased the proportion of CD4⁺IL-4⁺ cells from 4.7% to 9.3%; PG102T or PG102E reduced the number by more than 30%. In contrast, CD4⁺IFN- γ ⁺ cells were slightly increased from 7.6% to 9.2% or 9.6% when animals were treated with PG102T or PG102E, although not in a statistically significant manner. There was little change in the number of both cell types in DEX-administered animals. These results indicated that PG102T and PG102E might regulate the proportion of IL-4- and IFN- γ -producing T cells.

Next we analyzed the effects of PG102T and PG102E on the IgE-producing B cells. Compared with the DW treatment, PG102T or PG102E resulted in a decrease of about 40% or 55% in the number of CD19⁺IgE⁺ B cells. DEX decreased the number of such B cells by more than 60% (Fig 2, B). The confocal microscopic analysis additionally found that there was a significant reduction in the level of IgE production in any given B cell. The signal intensity of IgE in B cells was dramatically increased in OVA-sensitized mice, but it was greatly decreased when animals were treated with PG102T. The effect of PG102E appears to be more potent than the effect of PG102T because the level of fluorescence intensity was further decreased (Fig 2, C). These results indicated that PG102T and PG102E downregulated the IgE production within a given B cell, as well as the number

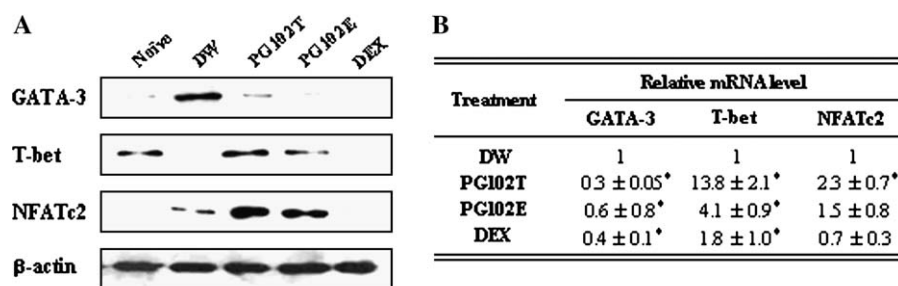


FIG 3. Effects of PG102T and PG102E on the expression of GATA-3, T-bet, and NFATc2 by means of Western blotting (A) and quantitative real-time PCR analyses (B). β -Actin was used as a loading control. The mRNA level for cellular genes was calculated as a relative value to reduced glyceraldehyde-phosphate dehydrogenase abundance. Results are expressed as means $\pm$ SEM from 3 independent experiments. * $P < .05$ vs DW-treated mice.

of IgE-producing B cells, ultimately resulting in the decrease in plasma IgE levels.

Effects of PG102T and PG102E on cellular transcription factors

To understand the molecular mechanisms underlying the biologic activities of PG102T and PG102E, we investigated whether these 2 preparations had any effects on cellular transcription factors involved in T_H1 and T_H2 pathways. This was because the effects of PG102T or PG102E on such multiple cytokines and IgE could more readily be explained if PG102, directly or indirectly, regulates a transcription factor or factors. It is well known that transcription factors, including GATA-3, T-bet, and NFATc2, play major roles in the balancing of T_H1 and T_H2 responses.^{6-9,18} Splenocytes isolated from OVA-sensitized mice were cultured for 2 days as described above and prepared for immunoblotting analysis. The protein level of GATA-3 was greatly decreased by both PG102T and PG102E, whereas that of T-bet and NFATc2 was upregulated (Fig 3, A). DEX suppressed all these transcription factors tested, as previously reported.¹⁹

The mRNA levels of respective transcription factors were determined by using a quantitative real-time PCR technique to verify at what level this regulation occurs (Fig 3, B). PG102T treatment decreased the mRNA level of GATA-3 by almost 3-fold, whereas it increased that of T-bet and NFATc2 by approximately 14-fold and 2-fold, respectively. It is interesting to note that PG102E, which showed higher biologic activity in terms of an IgE-inhibiting effect, changed the levels of GATA-3, T-bet, and NFATc2 to a lesser extent than PG102T. DEX lowered the mRNA levels of both GATA-3 and NFATc2 and slightly increased that of T-bet. These results indicated that PG102T and PG102E administration might control the production of T_H1 and T_H2 cytokines by regulating the level of transcription factors, such as GATA-3 and T-bet, at the RNA level.

DISCUSSION

The biologic activities of PG102T and PG102E observed in this study strongly indicate that the extracts from

A. arguta would be excellent candidates for potent and unique antiallergic agents. PG102T and PG102E not only inhibited the production of IgE in the LPS-stimulated U266B1 cells but also decreased the plasma level of IgE in OVA-sensitized mice. Because IgE is one of the central mediators responsible for the progression of allergic reactions,² these results indicate that PG102 might have an antiallergic activity by inhibiting IgE production from B cells, either directly or indirectly.

PG102 appears to have such biologic activity by modulating the T_H1 and T_H2 balance through the decrease in the level of selective T_H2 cytokines and the increase in that of T_H1 cytokines. The effects of PG102T and PG102E on cellular transcription factors, such as GATA-3 and T-bet, might explain how 2 preparations work at the molecular level. GATA-3 is known to strongly trans-activate the IL-5 promoter and the IL-4 enhancer element.^{6,7} T-bet is involved in the commitment of T_H1 cells by inducing the synthesis of IFN- γ in T_H1 cells.^{8,9} In recent studies it was also reported that T-bet regulates the isotype switching to IgG2a and the production of IFN- γ in B cells.²⁰ Our data are consistent with these known functions of respective transcription factors, namely downregulation of GATA-3-dependent expression of IL-4 and IL-5 and upregulation of T-bet-dependent production of IFN- γ and IgG2a.

(The possible action mechanism of PG102 is further discussed in the Online Repository in the online version of this article at www.jacionline.org.) Given the effects of PG102T or PG102E on the T_H1 and T_H2 systems, PG102 is thought to consist of several different biologically active compounds. (For discussion concerning the active compound or compounds of *A. arguta* and PG102, see the Online Repository in the online version of this article at www.jacionline.org.) Such complexity of herbal medicines and their extracts makes the standardization of herbal products, as well as the identification of active compounds, difficult. Therefore to obtain different PG102 preparations in a reliable manner, we devised *in vitro* bioassay systems to determine their specific bioactivity. The IL-4-based assay used in this study was sensitive and reproducible enough for us to calculate the specific activity of PG102. This IL-4-based assay is now being

used for the purification of active compounds, as well as the quality control of PG102 reagents used in a variety of experiments.

Our toxicity data indicate that PG102T is safe. In repeated dose toxicity trials, oral administration of PG102T (100, 300, 1000 mg/kg/d) for 4 weeks showed no visible adverse effects in rats. These results are in stark contrast with the data obtained from mice administered DEX (0.5 mg/kg/d), which showed severe reductions in body weights and masses of spleens (data not shown). Together with data on the various biologic activities of PG102T and PG102E, the extracts from *A arguta* might be potential candidates for natural and safe agents for various allergic disorders with a minimum risk of side effects. Considering the safety and biologic activity of PG102, further investigations into the identification of active compounds and other *in vivo* disease models are warranted.

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REFERENCES

1. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. *Nature* 1991;383:787-93.
2. Vercelli D. Immunoglobulin E and its regulators. *Curr Opin Allergy Clin Immunol* 2001;1:61-5.
3. Umetsu DT, DeKruyff RH. TH1 and TH2 CD4+ cells in human allergic diseases. *J Allergy Clin Immunol* 1997;100:1-6.
4. Coffman RL, Carty J. A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-gamma. *J Immunol* 1986;136:949-54.
5. Gavett SH, O'Hearn DJ, Li X, Huang SK, Finkelman FD, Wills-Karp M. Interleukin 12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice. *J Exp Med* 1995;182:1527-36.
6. Lee HJ, Takemoto N, Kurata H, Kamogawa Y, Miyatake S, O'Garra A, et al. GATA-3 induces T helper cell type 2 (Th2) cytokine expression and chromatin remodeling in committed Th1 cells. *J Exp Med* 2000;192:105-15.
7. Ting CN, Olson MC, Barton KP, Leiden JM. Transcription factor GATA-3 is required for development of the T-cell lineage. *Nature* 1996;384:474-8.
8. Lighvani AA, Frucht DM, Jankovic D, Yamane H, Aliberti J, Hissong BD, et al. T-bet is rapidly induced by interferon-gamma in lymphoid and myeloid cells. *Proc Natl Acad Sci U S A* 2001;98:15137-42.
9. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000;100:655-69.
10. Kato Y, Manabe T, Tanaka Y, Mochizuki H. Effect of an orally active Th1/Th2 balance modulator, M50367, on IgE production, eosinophilia, and airway hyperresponsiveness in mice. *J Immunol* 1999;162:7470-9.
11. Hofstra CL, Van Ark I, Hofman G, Kool M, Nijkamp FP, Van Oosterhout AJ. Prevention of Th2-like cell responses by coadministration of IL-12 and IL-18 is associated with inhibition of antigen-induced airway hyperresponsiveness, eosinophilia, and serum IgE levels. *J Immunol* 1998;161:5054-60.
12. Tomkinson A, Duez C, Cieslewicz G, Pratt JC, Joetham A, Shanafelt MC, et al. A murine IL-4 receptor antagonist that inhibits IL-4- and IL-13-induced responses prevents antigen-induced airway eosinophilia and airway hyperresponsiveness. *J Immunol* 2001;166:5792-800.
13. Kang HW, Yu KW, Jun WJ, Chang IS, Han SB, Kim HY, et al. Isolation and characterization of alkyl peroxy radical scavenging compound from leaves of *Laurus nobilis*. *Biol Pharm Bull* 2002;25(1):102-8.
14. Kim HM, Lee JH, Won JH, Park EJ, Chae HJ, Kim HR, et al. Inhibitory effect on immunoglobulin E production *in vivo* and *in vitro* by *Siegesbeckia glabrescens*. *Phytother Res* 2001;15:572-6.
15. Hirano T, Yamakawa N, Miyajima H, Maeda K, Takai S, Ueda A, et al. An improved method for the detection of IgE antibody of defined specificity by ELISA using rat monoclonal anti-IgE antibody. *J Immunol Methods* 1989;119:145-50.
16. Kyoko K, Yoshinao S, Yoko K, Masako M. Flow cytometric analysis of IL-4, IL-13 and IFN- γ expression in peripheral blood mononuclear cells and detection of circulating IL-13 in patients with atopic dermatitis provide evidence for the involvement of type 2 cytokines in the disease. *J Dermatol Sci* 2002;29:19-25.
17. Semper AE, Heron K, Woollard ACS, Kochan JP, Friedmann PS, Church MK, et al. Surface expression of Fc ϵ RI on Langerhans' cells of clinically uninvolved skin is associated with disease activity in atopic dermatitis, allergic asthma, and rhinitis. *J Allergy Clin Immunol* 2003;112:141-9.
18. Kiani A, Garcia-Cozar FJ, Habermann I, Laforsch S, Aebischer T, Ehninger G, et al. Regulation of interferon-gamma gene expression by nuclear factor of activated T cells. *Blood* 2001;98:1480-8.
19. Adcock IM. Glucocorticoid-regulated transcription factors. *Pulm Pharmacol Ther* 2001;14:211-9.
20. Gerth AJ, Lin L, Peng SL. T-bet regulates T-independent IgG2a class switching. *Int Immunol* 2003;15:937-44.