Allergy Asthma Immunol Res. 2020 Nov;12(6):949-963
https://doi.org/10.4168/aair.2020.12.6.949
pISSN 2092-7355·eISSN 2092-7363

Allergy, Asthma & Immunology Research AAIR

Original Article

Efficacy, Safety, and Immunomodulatory Effect of the Intramuscular Administration of Autologous Total Immunoglobulin G for Atopic Dermatitis: A Randomized Clinical Trial

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Received: Mar 30, 2020
Revised: Jun 3, 2020
Accepted: Jun 11, 2020

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ABSTRACT

Purpose: The management of patients with atopic dermatitis (AD) is often difficult. We hypothesized that repeated intramuscular administration of autologous total immunoglobulin G (IgG) could induce clinical improvement in patients with AD through immune modulation. This clinical trial was conducted to evaluate the efficacy, safety, and immunomodulatory effect of the intramuscular administration of autologous total IgG in patients with AD.

Methods: In this randomized, double-blind, placebo-controlled trial, 51 adolescent and adult patients with moderate-to-severe AD were randomized to receive 8 weekly intramuscular administrations of autologous total IgG 50 mg (n = 26) or saline (n = 25) over a 7-week period and were followed up to week 16. Changes in the clinical severity score (Eczema Area and Severity Index), affected body surface area, patient-reported Dermatology Life Quality Index (DLQI) score, laboratory biomarkers, and incidence of adverse events from baseline to week 16 were assessed.

Results: The intramuscular administration of autologous total IgG, compared with saline, decreased the clinical severity score (−64.8% vs. −20.3%, $P < 0.001$), reduced the affected body surface area (−53.9% vs. −19.1%, $P < 0.001$), improved the DLQI score (−35.4% vs. −14.4%, $P = 0.015$), increased serum interleukin-10 and interferon-γ levels ($P = 0.011$ and $P = 0.003$, respectively), and reduced the incidence of AD exacerbation (11.5% vs. 48.0%, $P = 0.004$) from baseline to week 16. No serious adverse events were observed.

Conclusions: The intramuscular administration of autologous total IgG provided clinical improvements and a systemic immunomodulatory effect in adolescent and adult patients with moderate-to-severe AD without significant side effects.

Trial Registration: Clinical Research Information Service Identifier: KCT0001597

Keywords: Atopic dermatitis; clinical trial; immunoglobulin G

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Trial Registration
Clinical Research Information Service
Identifier: KCT0001597

Disclosure
There are no financial or other issues that might lead to conflict of interest.

INTRODUCTION

Atopic dermatitis (AD) is a common chronic inflammatory skin disorder characterized by itching, dry skin, exudation, and frequent association with a personal or familial history of atopic diseases.[1-3]

The standard medical therapies using topical anti-inflammatory agents (corticosteroids and calcineurin inhibitors) and systemic immunosuppressants (corticosteroids, cyclosporine, and methotrexate, *etc.*) have limited clinical efficacy in patients with moderate-to-severe AD.[1] Current studies to develop new therapeutic modalities for AD focus on the monoclonal antibodies specifically blocking the function of T helper 2 (Th2) cytokines.[2] Recent randomized clinical studies showed that monoclonal antibodies to interleukin (IL)-4 receptor alpha or IL-13 induced significant clinical improvements in patients with moderate-to-severe AD.[4-7] However, clinical efficacies of the current medical therapies for AD are transient and incomplete.[1-3] Further development of a new therapeutic modality inducing a long-term treatment-free clinical improvement and modifying the disease course of AD is needed.

We searched for an empirically developed traditional therapy for AD that could be developed into a new therapeutic modality and found this possibility in autologous blood therapy (ABT) and autologous serum therapy (also known as autohemotherapy and autoserum therapy, respectively).[8-12] These simple treatment methods involve repeated administrations of a small amount (1–5 mL) of autologous blood or autologous serum to the same subjects by intramuscular injections, immediately after venous blood sampling.[8-12] These therapies have been used for the treatment of AD and chronic urticaria by physicians in many countries, including Europe, America, and Asia, for more than 100 years since they were first reported in 1913.[8-12] ABT has been reported as the most commonly practiced complementary and alternative medicine modality for AD.[13] Randomized, double-blind, placebo-controlled studies have demonstrated the favorable clinical efficacy of ABT and autologous serum therapy in patients with AD and chronic urticaria, respectively.[10,11] However, a therapeutic component of the blood mediating the clinical efficacy of ABT for AD and its therapeutic mechanism have not yet been identified. Thus, we attempted to identify the therapeutic component and mechanism of action. We hypothesized that the therapeutic component of ABT for AD is an autologous total immunoglobulin including pathogenic antibodies and that the therapeutic mechanism is an anti-idiotypic immunomodulation induced by the intramuscular administration of autologous total immunoglobulin.

To prove the concept, we evaluated the clinical efficacy, safety, and immunomodulatory effect of the intramuscular administration of autologous total immunoglobulin G (IgG) in 20 adult patients with severe AD as a pilot study.[14,15] In the study, 8 intramuscular administrations of autologous total IgG 50 mg over 4 weeks significantly decreased clinical severity scores and serum total IgE levels, and significantly increased the serum IL-10 and interferon-gamma (IFN-γ) levels in adult patients with severe AD at weeks 4, 8, and 12 compared to those at baseline, without serious adverse events.[15-17]

In this study, as a second trial to prove the concept, we performed a randomized, double-blind, placebo-controlled trial to evaluate the efficacy, safety, and immunomodulatory effect of the intramuscular administration of autologous total IgG in adolescent and adult patients with moderate-to-severe AD.

https://doi.org/10.4168/aair.2020.12.6.949

MATERIALS AND METHODS

Study design

A randomized, double-blind, placebo-controlled, parallel-group study was conducted at a single academic center (Ajou University Hospital, Suwon, Korea). Patients were recruited from April 2015 to November 2016 at an outpatient clinic. A 4-week screening and washout period was followed by a 7-week intervention period and a 9-week follow-up period (**Fig. 1A**).

This study was conducted in compliance with the guidelines for Good Clinical Practice and the Declaration of Helsinki, with approval from the institutional review board of Ajou University Hospital. All patients provided written informed consent. This clinical trial was registered in the Clinical Research Information Service of Korea, one of the primary registries in the World Health Organization international clinical trials registry platform (KCT0001597).

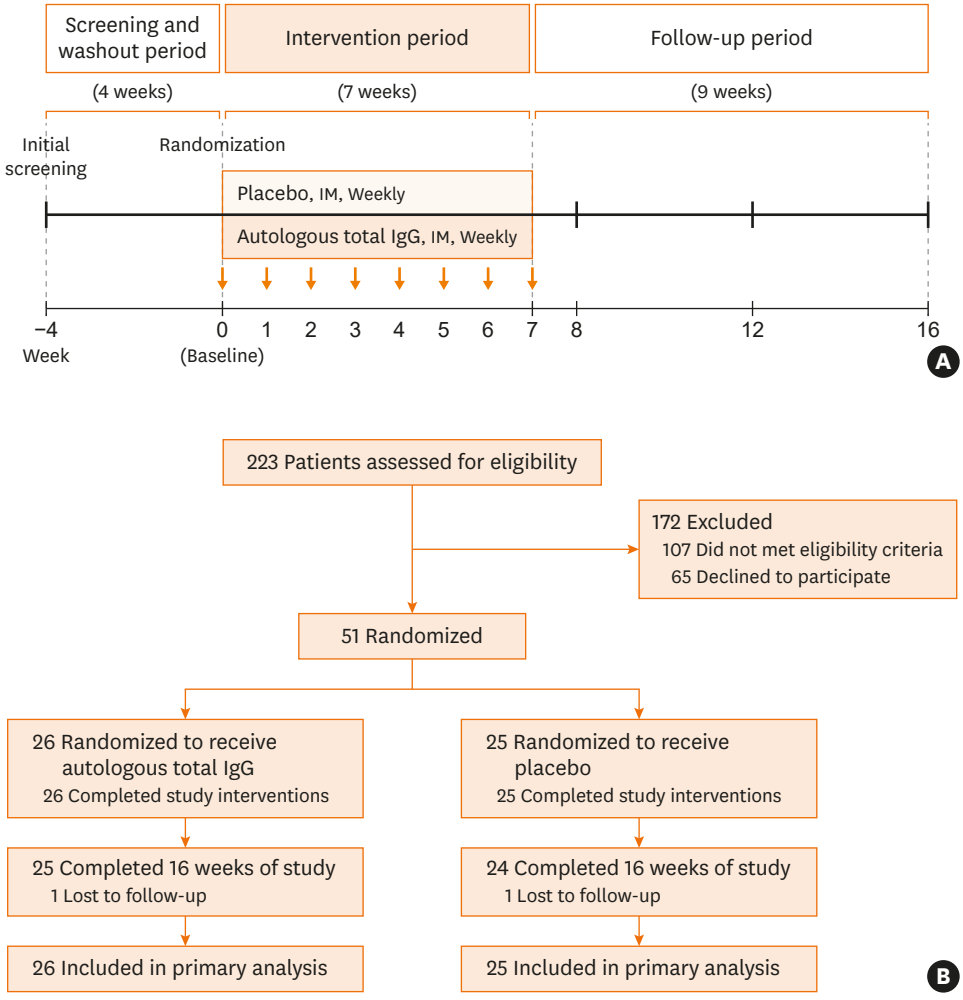

Fig. 1. The study design (A) and numbers of patients enrolled and included in the primary analysis (B). The arrows indicate the timing of the intramuscular injection.
IM, intramuscular injection; IgG, immunoglobulin G.

https://doi.org/10.4168/aair.2020.12.6.949

Patients

Eligible participants were adolescent and adult patients (age ≥ 13 years), with moderate-to-severe AD inadequately controlled by topical corticosteroids and/or topical calcineurin inhibitors, typical clinical features compatible with the diagnostic criteria for AD suggested by Hanifin and Rajka,[18] chronic AD for 3 years or longer, compatible with the criteria for autologous blood donation (hemoglobin level ≥ 11.0 g/dL and body weight ≥ 40 kg),[19] Investigator's Global Assessment (IGA) score of 3 (moderate) or higher (the IGA ranges from 0 [clear] to 5 [very severe]),[20] SCORing Atopic Dermatitis (SCORAD) score ≥ 25 (the SCORAD ranges from 0 to 103, with higher scores indicating greater clinical severity of AD),[21] and body surface area affected by AD ≥ 10% at the initial screening and baseline.

Exclusion criteria were as follows: other active skin diseases concomitant with AD; ultraviolet radiation or systemic immunomodulatory therapy (corticosteroids, cyclosporine, and methotrexate, *etc.*) within 8 weeks before randomization; use of topical corticosteroids or topical calcineurin inhibitors within 4 weeks before randomization; pregnancy; lactation; addiction to alcohol; and concomitant severe systemic diseases.

Preparation of autologous total IgG

At the initial screening visit (week −4), patients eligible for study participation underwent sampling of a 400 mL of autologous venous blood using a double blood bag containing anticoagulants. Approximately 500 mg of autologous total IgG (purity ≥ 97%) was aseptically purified from the autologous plasma by affinity chromatography using protein A beads as previously described.[15,22] The 2 mL of autologous total IgG (50 mg) were aliquoted into sterile glass vials. As a placebo, 2 mL of saline were prepared in the same sterile glass vials for each patient. The vials were stored at −20°C. The liquid solution containing autologous total IgG was colorless and visually indistinguishable from saline.

Randomization and blinding

The randomization list was developed by an independent statistician using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA), with a block size of 4. The randomization list was given to a research coordinator who did not contact the patients, and the randomization list was kept in a locked cabinet to ensure the concealment of the allocation. At the baseline visit (week 0), patients eligible for study participation were randomized to the placebo and autologous total IgG groups at a 1:1 ratio. The research coordinator replaced the labels of vials containing either autologous total IgG or saline as 'for injection' for each patient according to the randomization list. During the study intervention, the frozen vials for injection were delivered to a nurse by the research coordinator and thawed at room temperature. The nurse performed the intramuscular injections.

Investigators, patients, laboratory personnel, and the nurse in contact with the patients were masked to the intervention assignments throughout the study.

Interventions

Patients were randomized to receive weekly intramuscular injections of either autologous total IgG 50 mg (2 mL) or saline (2 mL) for 8 times over a 7-week period (from baseline to week 7) and were followed up until week 16.

During the study period (from week −4 to week 16), all drugs and procedures indicated for the treatment of AD were discontinued, with the exception of topical moisturizers, to exclude

the effects of concomitant medical therapies. Systemic corticosteroids were provided to patients as a rescue medication to control unacceptable symptoms of AD at the investigator's discretion. Study interventions were continued as scheduled, regardless of the use of rescue medication. Additionally, topical and systemic anti-infective agents for the treatment of bacterial or viral infections were provided to patients at the investigator's discretion.

Outcome measures

The primary endpoint was the percentage change in the clinical severity score as assessed by the Eczema Area and Severity Index (EASI),[23] which ranges between 0 and 72, with higher scores indicating greater severity, from baseline to week 16.

Secondary endpoints included the percentage changes from baseline to week 16 in the clinical severity score as assessed by the SCORAD[21] and the patient-reported Dermatology Life Quality Index (DLQI) score,[24] which ranges between 0 and 30, with higher scores indicating a lower quality of life. Assessments of clinical severity scores (IGA, EASI, and SCORAD), and DLQI score were made at the initial screening visit (week −4), weekly from week 0 (baseline) to week 8, and at weeks 12 and 16. Adverse events, vital signs, and clinical conditions were monitored to assess safety at each visit. Laboratory safety parameters, including a complete blood cell count, blood glucose, liver enzymes, total bilirubin, albumin, lactate dehydrogenase, urea, and creatinine levels, were analyzed at baseline and at week 8.

We performed *post hoc* analyses on primary and secondary endpoints to provide additional information on the clinical efficacy that was comparable to the efficacy endpoints presented in recent clinical trials on therapeutic monoclonal antibodies for AD.[4-6] The endpoints assessed included the proportions of patients achieving at least a 50% reduction in the EASI score, at least a 75% reduction in the EASI score, and a reduction of at least 2 points in the IGA score from baseline at week 16.

Data were collected by the investigators and the statistical analysis was performed by the independent statisticians.

Assessment of laboratory biomarkers

Venous blood samples were obtained at the initial screening visit (week −4), baseline (week 0), and weeks 4, 8, 12, and 16. Serum samples were stored at −20°C. The serum IL-10 and IFN-γ levels were measured by enzyme-linked immunosorbent assays using monoclonal antibody kits and standards (BD PharMingen, San Diego, CA, USA). The serum total IgE level was measured by an enzyme-linked immunosorbent assay using affinity-purified goat anti-human IgE antibodies (Vector Laboratories, Inc., Burlingame, CA, USA) as previously described.[25] The peripheral blood eosinophil count was measured by an automated hematology analyzer (Coulter Counter STKS; Beckman Coulter, Fullerton, CA, USA).

Flow cytometric analysis of IL-10- and IFN-γ-producing T cells

Peripheral blood mononuclear cells were isolated from the venous blood samples of three patients with AD who received 8 weekly intramuscular administrations of autologous total IgG 50 mg for 7 weeks at baseline and at week16. All three patients were primarily allocated to the placebo group in the randomized clinical trial. After the completion of the randomized clinical trial, these patients received intramuscular administrations of autologous total IgG in the same schedules and conditions of the randomized clinical trial. Flow cytometric analysis was performed as previously described.[26]

https://doi.org/10.4168/aair.2020.12.6.949

Statistical analysis

By our calculations, a sample size of 22 patients per group would provide the study with 90% power to detect a 40% difference between the study groups in the percentage change in the EASI score from baseline to week 16, assuming a standard deviation of 40% (with a 2-sided test and 0.05 significance level). Based on an expected dropout rate of 10%, the sample size was determined as 25 patients per group.

We performed an efficacy analysis using the intention-to-treat population, which included all randomized patients who were administered at least one study intervention. Continuous endpoints were analyzed using a mixed-effect model repeated measures to estimate the least squares (LS) means. In this model, no imputation for missing data was applied. A compound symmetry covariance matrix was used to model the within-patient errors. The model included fixed effects for treatment, week, and treatment-by-week interaction. Inter-group comparisons of the treatment effects by the mixed-effect model repeated measures were based on the LS mean changes (with 95% confidence interval [CI]) from baseline to week 16. Categorical variables were analyzed using Pearson's χ^2 test.

The Wilcoxon signed-rank test was used to analyze the within-group differences of the changes in laboratory parameters from baseline to week 16. The Mann-Whitney *U* test was used to analyze the inter-group differences of the changes in laboratory parameters from baseline to week 16. All analyses were 2-sided. A *P* value <0.05 was considered significant. Statistical analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Study patients

A total of 223 patients with AD were assessed for study eligibility. Among them, 52 adolescent and adult patients (aged ≥ 13 years) with moderate-to-severe AD were eligible for study participation based on the inclusion criteria at the initial screening visit. One patient was excluded at the baseline visit due to spontaneous clinical improvement (inclusion criteria violation). At the baseline visit, 51 patients (age range, 14–38 years; mean age, 25.6 years; 37 men [72.5%]) were randomized to receive 8 weekly intramuscular administrations of autologous total IgG 50 mg (n = 26) or saline (n = 25) over a 7-week period (**Fig. 1**). All 51 randomized patients completed the study interventions. One patient in the placebo group and one patient in the autologous total IgG group were lost to follow-up because of noncompliance (at weeks 12 and 16, respectively). The baseline demographic and clinical characteristics were similar in the placebo and autologous total IgG groups (**Table 1**).

Clinical efficacy endpoints

The LS mean percentage change in the EASI score from baseline to week 16 was −20.3% (95% CI, −32.0 to −8.7) in the placebo group and −64.8% (95% CI, −76.2 to −53.4) in the autologous total IgG group ($P < 0.001$) (**Table 2** and **Fig. 2A**). The LS mean percentage change in the SCORAD score from baseline to week 16 was −16.8% (95% CI, −24.4 to −9.2) in the placebo group and −37.8% (95% CI, −45.2 to −30.3) in the autologous total IgG group ($P < 0.001$) (**Table 2** and **Fig. 2B**). The LS mean percentage change in the DLQI score from baseline to week 16 was −14.4% (95% CI, −26.4 to −2.4) in the placebo group and −35.4% (95% CI, −47.2

Table 1. Baseline demographic and clinical characteristics of the patients

Characteristics	Placebo (n = 25)	Autologous total IgG (n = 26)
Age (yr)	26.8 (6.1)	24.5 (6.0)
Male sex	19 (76.0)	18 (69.2)
Body mass index (kg/m^2)	23.7 (4.4)	23.1 (3.9)
Duration of disease (yr)	21.0 (8.5)	19.9 (8.0)
IGA score		
3 (moderate)	5 (20.0)	2 (7.7)
4 (severe)	15 (60.0)	19 (73.1)
5 (very severe)	5 (20.0)	5 (19.2)
SCORAD score	77.5 (11.7)	79.4 (8.7)
Body surface area affected (%)	61.4 (14.7)	64.0 (14.8)
EASI score	37.3 (12.3)	40.5 (10.9)
DLQI score	18.2 (7.4)	17.6 (5.8)
Concomitant atopic diseases		
Asthma	7 (28.0)	9 (34.6)
Allergic rhinitis	18 (72.0)	21 (80.8)
Allergic conjunctivitis	8 (32.0)	8 (30.8)

Data are presented as mean (standard deviation) or number (%).
IGA, Investigator's Global Assessment; SCORAD, SCORing Atopic Dermatitis; EASI, Eczema Area and Severity Index; DLQI, Dermatology Life Quality Index; IgG, immunoglobulin G.

to −23.7) in the autologous total IgG group (P = 0.015). The LS mean percentage change in the affected body surface area from baseline to week 16 was −19.1% (95% CI, −29.3 to −8.9) in the placebo group and −53.9% (95% CI, −63.9 to −43.9) in the autologous total IgG group (P < 0.001) (**Table 2**).

The proportion of patients achieving at least a 50% reduction in the EASI score from baseline at week 16 was 29.2% (7/24 patients) in the placebo group and 72.0% (18/25 patients) in the autologous total IgG group (P = 0.003) (**Table 2**). The proportion of patients achieving at least a 75% reduction in the EASI score from baseline at week 16 was 12.5% (3/24 patients) in the placebo group and 48.0% (12/25 patients) in the autologous total IgG group (P = 0.007) (**Table 2**). The proportion of patients achieving a reduction of at least 2 points in the IGA score from baseline at week 16 was 25.0% (6/24 patients) in the placebo group and 56.0% (14/25 patients) in the autologous total IgG group (P = 0.027) (**Table 2**).

Table 2. Changes in clinical efficacy endpoints from baseline to week 16

Outcome	Placebo (n = 25)			Autologous total IgG (n = 26)			Difference in percentage change for autologous total IgG vs placebo, LS mean (95% CI)	*P* value
	Baseline, mean (SD)	Week 16, mean (SD)	Percentage change from baseline, LS mean (95% CI)	Baseline, mean (SD)	Week 16, mean (SD)	Percentage change from baseline, LS mean (95% CI)		
EASI score	37.3 (12.3)	29.6 (18.9)	−20.3% (−32.0 to −8.7)	40.5 (10.9)	14.1 (10.2)	−64.8% (−76.2 to −53.4)	−44.4% (−60.7 to −28.2)	< 0.001
SCORAD score	77.5 (11.7)	64.8 (22.2)	−16.8% (−24.4 to −9.2)	79.4 (8.7)	49.3 (18.6)	−37.8% (−45.2 to −30.3)	−21.0% (−31.6 to −10.3)	< 0.001
DLQI score	18.2 (7.4)	16.2 (9.4)	−14.4% (−26.4 to −2.4)	17.6 (5.8)	11.2 (7.2)	−35.4% (−47.2 to −23.7)	−21.0% (−37.8 to −4.2)	0.015
Body surface area affected (%)	61.4 (14.7)	50.7 (26.3)	−19.1% (−29.3 to −8.9)	64.0 (14.8)	29.6 (18.1)	−53.9% (−63.9 to −43.9)	−34.8% (−49.1 to −20.5)	< 0.001
EASI-50 at week 16*	7/24 (29.2)			18/25 (72.0)			-	0.003
EASI-75 at week 16*	3/24 (12.5)			12/25 (48.0)			-	0.007
Reduction in IGA score ≥ 2 points from baseline at week 16*	6/24 (25.0)			14/25 (56.0)			-	0.027

Data are presented as mean (standard deviation, SD) or number (%).
EASI, Eczema Area and Severity Index; SCORAD, SCORing Atopic Dermatitis; DLQI, Dermatology Life Quality Index; IGA, Investigator's Global Assessment; LS, least squares; CI, confidence interval; IgG, immunoglobulin G; EASI-50, proportion of patients achieving at least a 50% reduction in the EASI score from baseline; EASI-75, proportion of patients achieving at least a 75% reduction in the EASI score from baseline.
*The number of patients with EASI and IGA scores at week 16 was 24 for the placebo group and 25 for the autologous total IgG group.

https://doi.org/10.4168/aair.2020.12.6.949

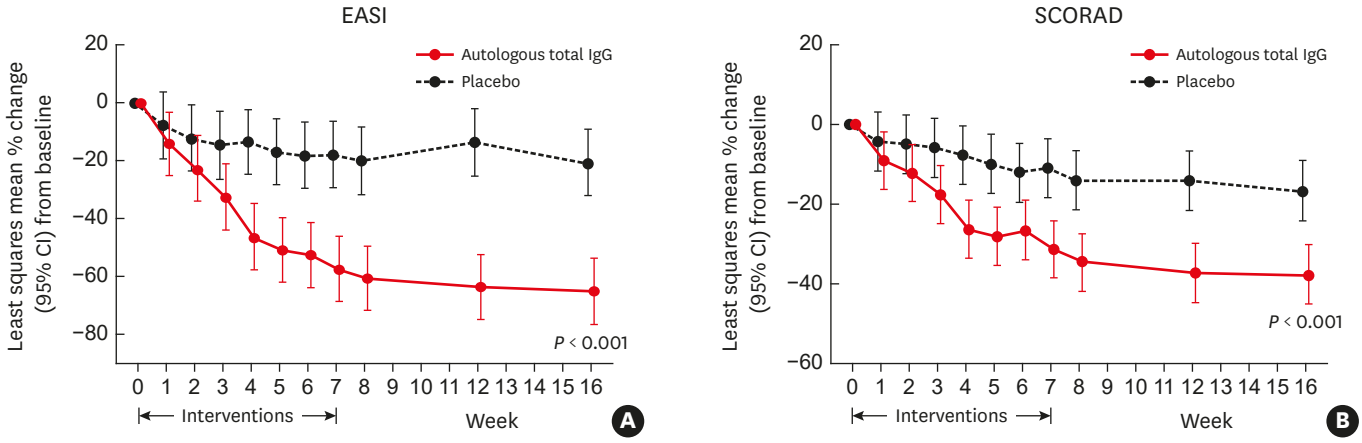

Fig. 2. Changes in clinical severity scores of atopic dermatitis. The least squares mean percentage changes in the clinical severity scores of atopic dermatitis assessed by the EASI (A) and SCORAD (B). Error bars indicate 95% CIs. The *P* value comparisons are for week 16 and were determined by a mixed-effect model repeated measures.
IgG, immunoglobulin G; CI, confidence interval; EASI, Eczema Area and Severity Index; SCORAD, SCORing Atopic Dermatitis.

Safety

No serious adverse events or death were reported. Overall, 60.8% (31/51 patients) of the randomized patients reported at least one adverse event (**Table 3**). The most common adverse event was AD exacerbation, which was more frequently reported in the placebo group (48.0%; 12/25 patients) than in the autologous total IgG group (11.5%; 3/26 patients) ($P = 0.004$). The proportion of patients who received a systemic corticosteroid as a rescue medication to control unacceptable exacerbation of AD was 28.0% (7/25 patients) in the placebo group and 7.7% (2/26 patients) in the autologous total IgG group ($P = 0.075$, Fisher's exact test). Other common adverse events included nasopharyngitis, bacterial skin infection, and upper respiratory tract infection, which were reported at similar frequencies in the placebo and autologous total IgG groups. There were no significant changes in the laboratory parameters of liver and renal functions and complete blood cell counts from baseline to week 8.

Table 3. Adverse events

Events	Placebo (n = 25)	Autologous total IgG (n = 26)
Total No. of adverse events	32	19
Patients with 1 ≥ adverse event	18 (72.0)	13 (50.0)
Patients with 1 ≥ serious adverse event	0	0
Patients with an adverse event leading to withdrawal from intervention	0	0
Exacerbation of atopic dermatitis	12 (48.0)	3 (11.5)
Nasopharyngitis	4 (16.0)	3 (11.5)
Bacterial skin infection	4 (16.0)	3 (11.5)
Upper respiratory tract infection	3 (12.0)	2 (7.7)
Headache	2 (8.0)	1 (3.8)
Eczema herpeticum	2 (8.0)	0
Herpes simplex	1 (4.0)	1 (3.8)
Urticaria	1 (4.0)	0
Conjunctivitis	1 (4.0)	0
Diarrhea	1 (4.0)	0
Myringitis	1 (4.0)	0
Nasal congestion	0	1 (3.8)
Injection-site reaction	0	5 (19.2)

Data are presented as number (%).
IgG, immunoglobulin G.

https://doi.org/10.4168/aair.2020.12.6.949

Changes in serum IL-10 and IFN-γ levels

The serum IL-10 and IFN-γ levels were significantly increased at weeks 4, 8, 12 and 16 compared to baseline in the autologous total IgG group ($P < 0.05$), while no significant differences were found in the placebo group (**Fig. 3A and B**). Changes in serum IL-10 and IFN-γ levels from baseline were significantly higher in the autologous total IgG group than in the placebo group at weeks 4, 8, 12, and 16 ($P < 0.05$) (**Fig. 3C and D**).

Changes in serum total IgE level and peripheral blood eosinophil count

The serum total IgE level was significantly decreased at weeks 4, 8, 12 and 16 compared to baseline in the autologous total IgG group ($P < 0.05$) (**Fig. 4A**), while no significant differences were found in the placebo group ($P > 0.05$). The change in serum total IgE level from baseline was significantly higher in the autologous total IgG group than in the placebo group at week 8 ($P < 0.05$). The peripheral blood eosinophil count was significantly decreased at week 16 compared to baseline in the placebo group ($P < 0.05$) (**Fig. 4B**), while no significant differences were found in the autologous total IgG group ($P > 0.05$). There were no significant

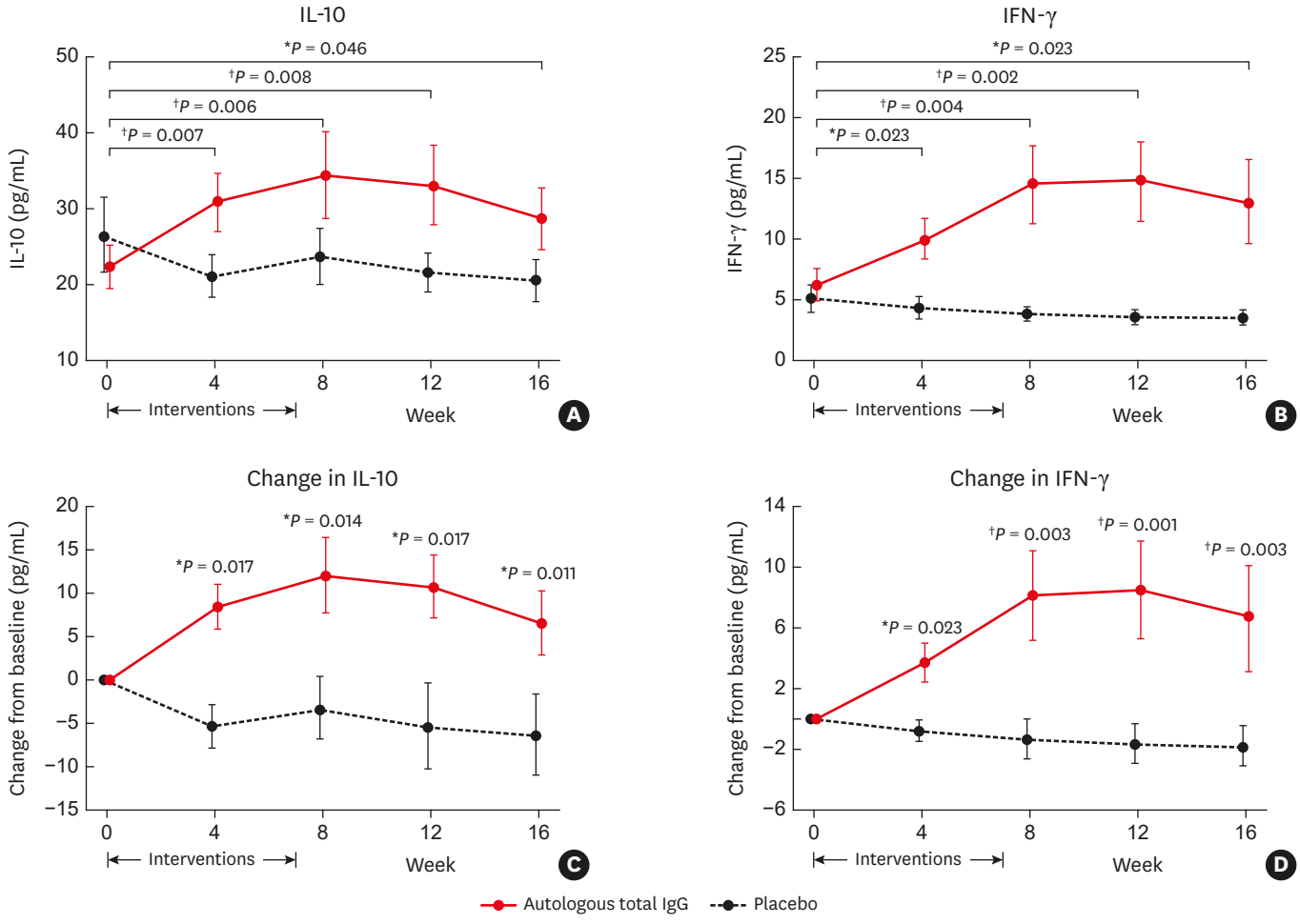

Fig. 3. Changes in serum IL-10 and IFN-γ levels. Serum levels of IL-10 and IFN-γ (A, B), and changes in the serum levels of IL-10 and IFN-γ from baseline (C, D). Data are presented as the mean and standard error of the mean. Within-group differences for the comparison with baseline (week 0) were analyzed by the Wilcoxon signed-rank test (A, B). Inter-group differences were analyzed by the Mann-Whitney *U* test (C, D).
IgG, immunoglobulin G; IL-10, interleukin-10; IFN-γ, interferon-gamma.
*$P < 0.05$; †$P < 0.01$.

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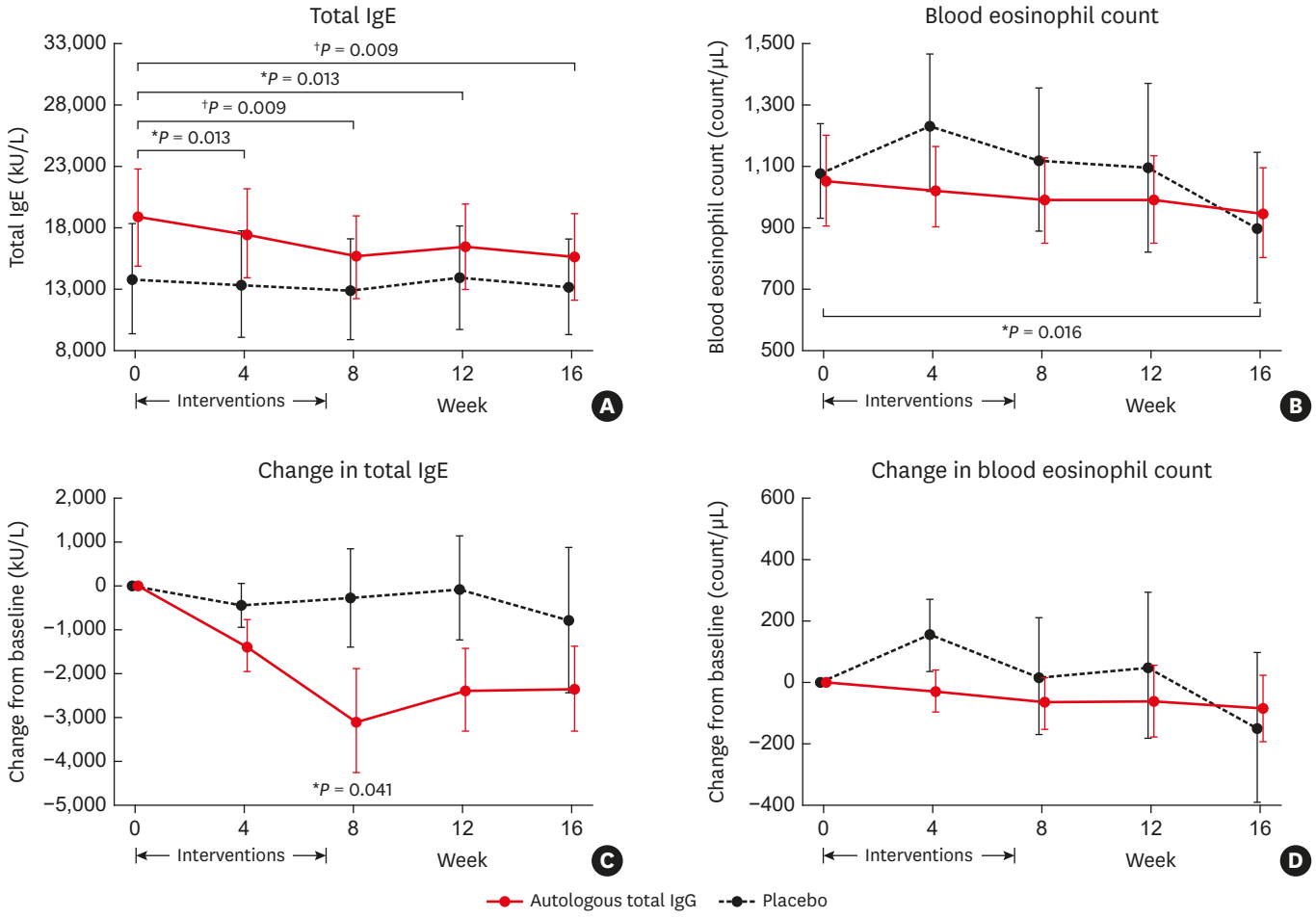

Fig. 4. Changes in serum total IgE level and peripheral blood eosinophil count. Serum total IgE level and peripheral blood eosinophil count (A, B) and changes in serum total IgE level and peripheral blood eosinophil count from baseline (C, D). Data are presented as mean and standard error of the mean. Within-group differences for the comparison with baseline (week 0) were analyzed by the Wilcoxon signed-rank test (A, B). Inter-group differences were analyzed by the Mann-Whitney *U* test (C, D).
IgE, immunoglobulin E.
$^{*}P < 0.05$; $^{\dagger}P < 0.01$.

inter-group differences in the changes in the peripheral blood eosinophil count from baseline at weeks 4, 8, 12, and 16 ($P > 0.05$).

Flow cytometric analysis

The proportions of cells with intracellular IL-10 and IFN-γ in the peripheral blood $CD3^+$ T cells and $CD4^+$ T cells were increased at week 16 compared to baseline in three patients with AD who received 8 weekly intramuscular administrations of autologous total IgG 50 mg for 7 weeks (**Table 4** and **Fig. 5**).

Table 4. The proportions of cells with intracellular IL-10 and IFN-γ in the peripheral blood $CD3^+$, $CD4^+$, and $CD8^+$ T cells in three patients with atopic dermatitis who received 8 weekly intramuscular administrations of autologous total IgG 50 mg for 7 weeks

Patient No.	IL-10$^+$ cells in CD3$^+$ T cells (%)		IL-10$^+$ cells in CD4$^+$ T cells (%)		IL-10$^+$ cells in CD8$^+$ T cells (%)		IFN-γ$^+$ cells in CD3$^+$ T cells (%)		IFN-γ$^+$ cells in CD4$^+$ T cells (%)		IFN-γ$^+$ cells in CD8$^+$ T cells (%)	
	Baseline	Week 16	Baseline	Week 16	Baseline	Week 16	Baseline	Week 16	Baseline	Week 16	Baseline	Week 16
32	0.1	1.7	0.1	1.8	0	0.7	0.5	32.7	0.7	26.6	0.8	18.6
49	0.1	1.1	0.2	0.9	0.2	0.1	0.1	4.7	0.3	3.0	0.4	3.8
51	0.2	0.5	0.1	0.3	0.2	0.3	0.2	6.6	0.1	7.8	0.4	17.6

IgG, immunoglobulin G; IL-10, interleukin-10; IFN-γ, interferon-gamma.

https://doi.org/10.4168/aair.2020.12.6.949

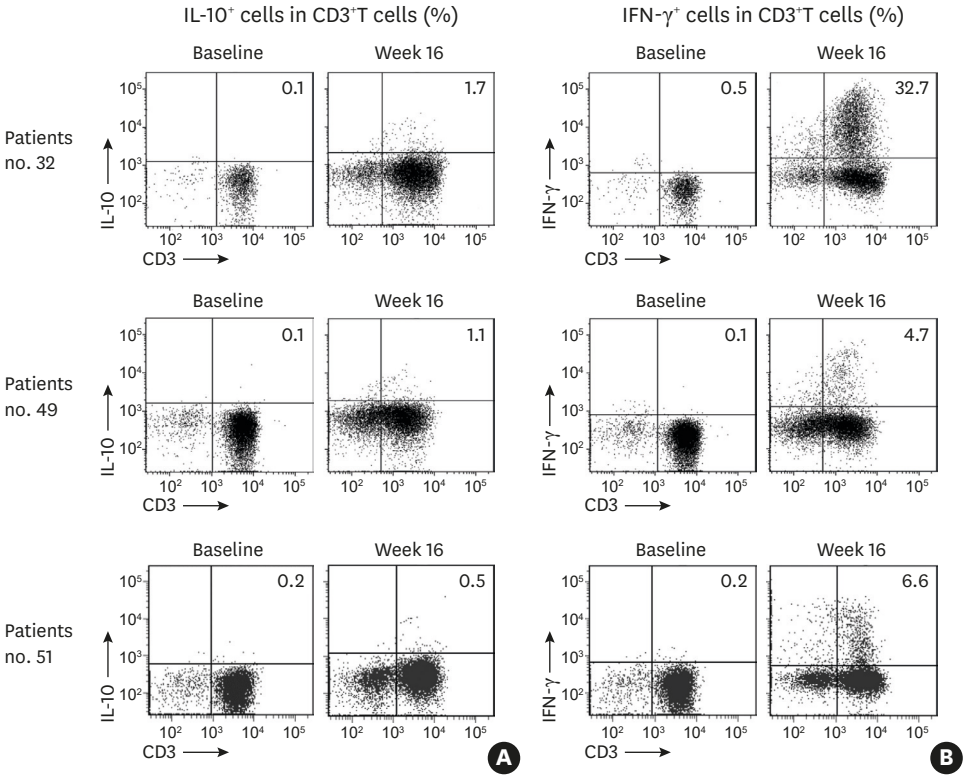

Fig. 5. Flow cytometric analysis of IL-10- and IFN-γ-producing T cells. The proportions of cells with intracellular IL-10 (A) and IFN-γ (B) in the peripheral blood CD3+ T cells were analyzed at baseline (week 0) and at week 16 in three patients with atopic dermatitis who received 8 weekly intramuscular administrations of autologous total IgG 50 mg for 7 weeks.
IgG, immunoglobulin G; IL-10, interleukin-10; IFN-γ, interferon-gamma.

DISCUSSION

This is the first randomized clinical trial in humans to evaluate the efficacy, safety, and immunomodulatory effect of the intramuscular administration of autologous total IgG. Our results demonstrated that intramuscular administration of autologous total IgG could provide clinical improvements in adolescent and adult patients with moderate-to-severe AD. This favorable clinical efficacy was supported by greater improvements in clinical severity scores (EASI and SCORAD) and quality of life score with autologous total IgG compared to the placebo. In this study, the clinical response rate (expressed as the EASI-50 response rate in week 16) of the 8 weekly intramuscular administrations of autologous total IgG 50 mg (total 400 mg of IgG) was 72%. This result is comparable to those EASI-50 response rates at week 16 of continuous weekly subcutaneous administrations of 300 mg of IgG monoclonal antibody to the IL-4 receptor alpha (after the first loading dose of 600 mg at baseline) for 16 weeks (total 4,800 mg of IgG) in two randomized clinical trials in patients with moderate-to-severe AD (83% in 63 patients and 61% in 223 patients in those studies).[5,6] However, the number of patients who received intramuscular administration of autologous total IgG in this study was relatively small (26 patients). Therefore, further clinical trials with larger numbers of patients with AD are needed to evaluate the clinical efficacy of intramuscular administration of autologous total IgG.

https://doi.org/10.4168/aair.2020.12.6.949

The clinical efficacies of current medical therapies for AD are transient and incomplete.[3,27] Patients with AD and their family members frequently attempt unproven treatment methods to achieve a cure or long-term treatment-free clinical remission, with disappointing results.[3] Development of a new therapeutic modality modifying the long-term clinical course of AD is needed to solve this problem. The present study showed clinical improvements lasting for at least 9 weeks after the completion of 8 weekly intramuscular administrations of autologous total IgG for 7 weeks in patients with AD. In our previous report, a long-term clinical improvement for more than 36 weeks was observed in two of three patients with severe AD who received 8 intramuscular administrations of autologous total IgG 50 mg for 4 weeks and were followed up for 2 years.[16] Further clinical trials with longer intervention and observation period are needed to evaluate a duration of clinical improvement lasting after completion of intramuscular administrations of autologous total IgG.

In this study, an exacerbation of AD was the most common adverse event and was more frequently observed in the placebo group than in the autologous total IgG group. No serious adverse events were reported. These results support the safety of the intramuscular administration of autologous total IgG in patients with AD.

Immune dysfunction, with decreased regulatory T-cell function (reflected by the decreased production of IL-10 from T cells)[28,29] and excessive activation of Th2 cells producing IL-4 and IL-13, seems to play a key role in the pathogenesis of AD.[30] However, the detailed characteristics of immune dysfunction are heterogeneous among patients with AD.[30,31] Therefore, a personalized immunomodulatory therapy to restore regulatory T-cell function and Th1/Th2-cell balance might be a reasonable approach to induce clinical improvements in patients with AD.[1,3] This study showed that intramuscular administration of autologous total IgG significantly increased serum IL-10 and IFN-γ levels in patients with AD. These systemic immunomodulatory effects cannot be simply explained by the passive supplementation of a small amount (50 mg) of autologous total IgG to the same subjects with AD. Furthermore, the proportions of IL-10- and IFN-γ-producing cells in the peripheral blood $CD4^+$ T cells were increased after the intramuscular administration of autologous total IgG in patients with AD. These results suggest that the activation of regulatory T cells producing IL-10 and Th1 cells producing IFN-γ, which were induced by the intramuscular administration of autologous total IgG, provided a systemic immunomodulatory effect.

The type of immune response (Th1 or Th2 response) provoked by a specific antigen is determined by the antigen dose and route of administration.[32,33] A low dose of an allergen (such as pollens or house dust mites) administered to the respiratory mucosa or skin induces an allergic reaction (Th2 immune response) in patients with allergic diseases.[34] However, repeated subcutaneous injections of a high dose of purified allergen (allergen immunotherapy) induce a Th1 immune response and reduce clinical symptoms in patients with allergic diseases.[35] We speculate that intramuscularly administered high doses of purified autologous total IgG may induce an immunomodulatory effect in patients with AD by changing the antigen dose, purity, and type of contacting antigen-presenting cells (versus its natural presence in the blood circulation). However, further studies are necessary to define the detailed mechanism for immunomodulation induced by the intramuscular administration of autologous total IgG (especially on the anti-idiotypic immunomodulation).

This clinical trial has several limitations, including its single-center design, relatively small number of patients, and short intervention duration. Further clinical trials with a larger

sample size and a longer intervention duration are needed to evaluate the long-term clinical usefulness of this approach.

Recent industrial developments in the production of therapeutic monoclonal antibodies can provide an automated purification of total IgG from plasma.[36] This methodological solution might be useful for the clinical application of the intramuscular administration of autologous total IgG in patients with AD.

In conclusion, the intramuscular administration of autologous total IgG provided clinical improvements and a systemic immunomodulatory effect in adolescent and adult patients with moderate-to-severe AD.

ACKNOWLEDGMENTS

We thank the patients for their participation and commitment. We also thank the following persons for their review of the manuscript and helpful comments: Hyun Young Lee, Ph.D., Clinical Trial Center, Ajou University Medical Center, Suwon, Korea; and Seungsoo Sheen, M.D., M.S., Department of Pulmonary and Critical Care Medicine, Ajou University School of Medicine, Suwon, Korea. We thank Jee Hyun Lee, R.N., for her excellent help in the sampling of venous blood from the patients.

The corresponding author is indebted to Prof. Chein-Soo Hong (Division of Allergy and Immunology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea) for his mentorship and encouragement in realizing this project.

This work was supported by National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No.2014R1A2A1A11049386).

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