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Preliminary report

Immunomodulatory effects induced by intramuscular administration of autologous total immunoglobulin G in patients with atopic dermatitis

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ABSTRACT

Background: Polyvalent human immunoglobulin G (IgG) preparations produced from the plasma pools of healthy blood donors have been used for the treatment of various autoimmune diseases and allergic diseases because of their anti-inflammatory and immunomodulatory effects. We hypothesized that intramuscular administration of autologous total IgG would induce immunomodulatory effects in patients with allergic diseases, based on the clinical efficacy of autologous blood therapy in patients with atopic dermatitis (AD).

Methods: Sixteen adult AD patients with IgE-mediated sensitization to the house dust mite (*Dermatophagoides farinae*) received intramuscular injections of 50 mg autologous total IgG twice a week for 4 weeks. The serum levels of IgE, IgG, and IgG4 antibodies to the recombinant group 2 major allergen of *Dermatophagoides farinae* (Der f 2) and serum levels of interleukin (IL)-10, IL-4, IL-12, and interferon gamma (IFN-γ) were measured by enzyme-linked immunosorbent assay at baseline and at weeks 4, 8, and 12.

Results: The serum level of IgE antibodies to Der f 2 was significantly decreased at 12 weeks compared with baseline ($p < 0.005$). The serum levels of IgG and IgG4 antibodies to Der f 2 were significantly increased at 4, 8, and 12 weeks compared with baseline ($p < 0.05$). The serum levels of IL-10 and IFN-γ were significantly increased at 4, 8, and 12 weeks compared with baseline ($p < 0.05$). There were no significant differences in the serum levels of IL-4 or IL-12 before and after intramuscular administrations of autologous total IgG ($p > 0.05$).

Conclusion: Intramuscular administration of autologous total IgG induced anti-allergic immunomodulatory effects in AD patients. Further studies are required to evaluate the detailed immunological mechanism underlying these effects.

1. Introduction

Polyvalent human immunoglobulin G (IgG) preparations produced from the plasma pools of multiple healthy human blood donors have been used as a standard treatment of primary immunodeficiency diseases associated with decreased production of immunoglobulin [1]. Polyvalent human IgG preparations have also has been used for the treatment of various autoimmune diseases and allergic diseases because of their anti-inflammatory and immunomodulatory effects [1]. The immunomodulatory mechanism of polyvalent human IgG preparations is not completely understood [1,2]. Natural IgG antibodies to the antigen-binding sites (idiotypes) of pathogenic antibodies (IgG autoantibodies or IgE antibodies) in human IgG preparations have been suggested to exert immunomodulatory effects by neutralizing and suppressing pathogenic antibodies [1]. This mechanism can be interpreted as restoration of idiotype-anti-idiotype immunoregulatory networks and immune homeostasis by passive administration of IgG anti-idiotype antibodies from healthy human blood donors to patients with autoimmune diseases and allergic diseases [1,2]. Alternatively, we hypothesize that active induction of an anti-idiotype immune response to pathogenic antibodies by immunization with autologous total immunoglobulin containing pathogenic antibodies can be another approach to restore immune homeostasis in patients with autoimmune diseases and allergic diseases.

The aim of this study, an evaluation of immunomodulatory effects induced by intramuscular administration of autologous total IgG in patients with atopic dermatitis (AD), originated from our hypothesis on the therapeutic mechanism of autologous blood therapy (ABT). ABT is a common alternative treatment for AD and chronic urticaria that has been practiced for > 100 years by physicians in Europe, South America, and Japan since its first report in 1913 [3]. ABT involves repeated administrations of small amounts of autologous whole blood (1–10 mL) by intramuscular injection immediately after sampling of venous blood [4]. The clinical efficacy of ABT has been demonstrated in patients with AD and chronic urticaria in randomized controlled studies [4,5]. Recently, the clinical efficacy of intramuscular administration of

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autologous serum (autologous serum therapy) was demonstrated in 111 patients with chronic urticaria in a randomized double-blind placebo-controlled study [6]. Important advantages of ABT include its historically proven clinical efficacy and clinical safety; there have been few reports of side effects [6]. However, the lack of knowledge regarding the blood component responsible for the therapeutic efficacy and mechanism of action is a major disadvantage of ABT.

We hypothesized that the blood component responsible for the therapeutic efficacy of ABT is an immunoglobulin, and that the therapeutic mechanism of ABT involves induction of anti-idiotype immunomodulatory effects by intramuscular administration of the autologous total immunoglobulin. We previously attempted to confirm this hypothesis by demonstrating the superior clinical efficacy of intramuscular administration of an autologous immunoglobulin-enriched plasma fraction compared with autologous plasma in adult AD patients [7]. We also performed an uncontrolled pilot clinical study to evaluate the efficacy and safety of intramuscular administration of autologous total IgG in 20 adult AD patients [8–10]. In these previous studies, intramuscular administration of 50 mg autologous total IgG twice a week for 4 weeks induced significant clinical improvements and decreases in the serum total IgE concentration in AD patients [8–10]. Long-term clinical improvements and decreases in serum total IgE concentrations lasting > 36 weeks were observed in 2 of 3 severe AD patients who were followed up for > 2 years after 4 weeks of intramuscular administrations of autologous total IgG in the previous report [10].

In this study, we analyzed changes in the serum levels of allergen-specific IgE and IgG antibodies and cytokines in AD patients with IgE-mediated sensitization to the house dust mite (HDM) before and after intramuscular administration of autologous total IgG, to evaluate the immunomodulatory effects induced by intramuscular administration of autologous total IgG in AD patients.

2. Materials and methods

2.1. Patients

The frozen stored serum samples from 16 adult AD patients with IgE-mediated sensitization to HDM (*Dermatophagoides farinae; D. farinae*) among the 17 adult AD patients who participated in our previous study were used in this study (one patient without an HDM allergy was excluded) [9]. This study was approved by the local institutional review board. All patients provided written informed consent.

2.2. Materials

Venous blood (400 mL) was collected at the initial visit (week − 4) using a double blood bag system (Green Cross PBM, Seoul, Korea) containing an anticoagulant. The venous blood was separated into packed red blood cells and plasma by centrifugation at 3500 rpm and 4 °C for 10 min. The separated autologous plasma (~200 mL) was stored at − 20 °C. Autologous total IgG (purity ≥ 97%) was aseptically purified from autologous plasma by affinity chromatography using Protein A beads, as described previously [8–10]. The aseptically purified autologous total IgG solution was tested for bacterial contamination using an endotoxin assay kit (Associates of Cape Cod Inc., Falmouth, MA, USA). The autologous total IgG solution was aliquoted into sterile glass vials and stored at − 20 °C.

2.3. Intramuscular administration of autologous total IgG and timing of serum sampling

The patients received intramuscular injections of 50 mg autologous total IgG twice a week for 4 weeks (eight injections total) between baseline (week 0) and 4 weeks (Fig. 1). The serum samples were collected at the initial visit (week − 4), at baseline (week 0), and at weeks 4, 8 and 12.

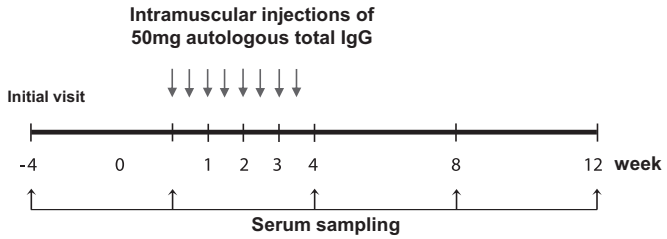


Fig. 1. Summary of the study design.

2.4. Measurement of serum levels of IgE, IgG, and IgG4 antibodies to HDM

The serum levels of IgE, IgG, and IgG4 antibodies to whole body extract (WBE) of *D. farinae* and the recombinant group 2 major allergen of *D. farinae* (Der f 2) (Prolagen, Seoul, Korea) were measured by enzyme-linked immunosorbent assay (ELISA) as described previously [11–13]. Serum levels of IgE antibodies to HDM were expressed as kU/L using an IgE standard (Phadia US, Portage, MI, USA). Serum levels of IgG and IgG4 antibodies to HDM were expressed in arbitrary units using high-titer serum as the standard.

2.5. Measurement of serum levels of IgG antibodies to other antigens

The serum levels of IgG antibodies to pneumococcal capsular polysaccharide antigens (Pneumo 23®, Sanofi Pasteur, Lyon, France), influenza antigens (Fluarix Tetra®, GlaxoSmithKline, Dresden, Germany), bovine thyroglobulin (Sigma, St Louis, MO, USA), and bovine thymus extract (Immunovision, Springdale, AR, USA) were measured by ELISA as described previously [14–18]. Serum levels of IgG antibodies to those antigens were expressed in arbitrary units using high-titer serum as the standard.

2.6. Measurement of serum cytokine levels

The serum concentrations of interleukin (IL)-10, IL-4, IL-12, and interferon gamma (IFN-γ) were measured by ELISA using monoclonal antibody kits and standards (BD PharMingen, San Diego, CA, USA) according to the manufacturer's instructions.

2.7. Statistical analysis

Data are expressed as means ± standard error of the mean (SEM). The statistical significance of the differences in values before and after intramuscular administration of autologous total IgG was analyzed using the Wilcoxon signed-rank test. A p-value < 0.05 was considered to indicate statistical significance.

3. Results

3.1. Changes in serum levels of IgE, IgG and IgG4 antibodies to HDM

The serum levels of IgE antibodies to WBE of *D. farinae* and Der f 2 were significantly decreased at 12 weeks compared with baseline ($p < 0.005$, Fig. 2). The serum levels of IgG antibodies to WBE of *D. farinae* were significantly increased at 8 and 12 weeks compared with baseline ($p < 0.05$, Fig. 3A). The serum levels of IgG antibodies to Der f 2 were significantly increased at 4, 8, and 12 weeks compared with baseline ($p < 0.05$, Fig. 3B). The serum levels of IgG4 antibodies to WBE of *D. farinae* were significantly increased at 4 and 12 weeks compared with baseline ($p < 0.05$, Fig. 3C). The serum levels of IgG4 antibodies to Der f 2 were significantly increased at 4, 8, and 12 weeks compared with baseline ($p < 0.05$, Fig. 3D). There were no significant

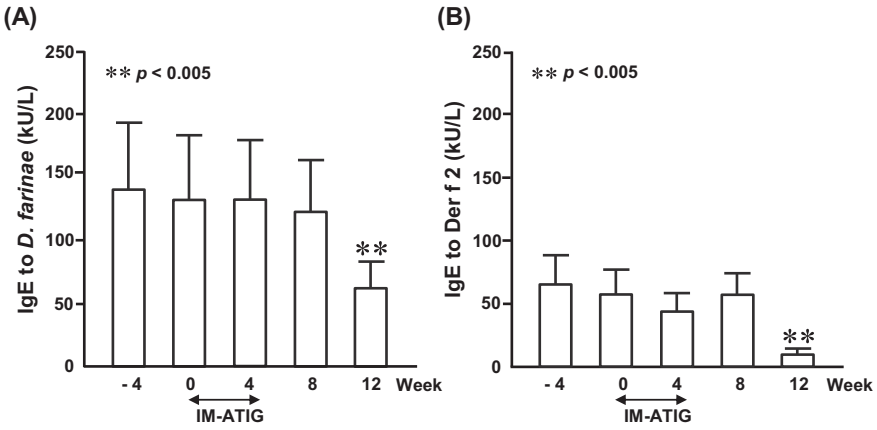


Fig. 2. Changes in serum levels of IgE antibodies to house dust mite allergens (whole body extract of *D. farinae* and recombinant Der f 2 allergen) in 16 adult patients with atopic dermatitis who received intramuscular administrations of 50 mg autologous total IgG (IM-ATIG) twice a week for 4 weeks. Data are means ± SEM. $^{**}p < 0.005$ compared with baseline (Wilcoxon signed-rank test).

differences in the serum levels of IgE, IgG, or IgG4 antibodies to WBE of *D. farinae* or Der f 2 between the initial visit (week − 4) and baseline (week 0) ($p > 0.05$, Figs. 2 and 3).

3.2. Changes in the serum levels of IgG antibodies to other antigens

There were no significant differences in the serum levels of IgG antibodies to pneumococcal capsular polysaccharide antigens, influenza antigens, bovine thyroglobulin, or bovine thymus extract (extractable nuclear antigens) before and after intramuscular administration of autologous total IgG ($p > 0.05$) (Fig. 4).

3.3. Changes in the serum levels of cytokines

The serum levels of IL-10 and IFN-γ were significantly increased at 4, 8, and 12 weeks compared with baseline ($p < 0.05$) (Fig. 5). There were no significant differences in the serum levels of IL-4 or IL-12 before and after intramuscular administration of autologous total IgG ($p > 0.05$) (Fig. 5).

4. Discussion

In this study, intramuscular administration of autologous total IgG induced significant anti-allergic immunomodulatory effects in AD patients. Although there have been numerous studies on the immunomodulatory effects of polyvalent IgG preparations from healthy blood donors in human subjects with allergic and autoimmune diseases [2], this study demonstrated anti-allergic immunomodulatory effects of intramuscular administration of autologous total IgG in human subjects. These anti-allergic immunomodulatory effects of autologous total IgG can be useful in the treatment of patients with allergic diseases.

Intramuscular administration of autologous total IgG induced significant decreases in serum levels of allergen-specific IgE antibodies and significant increases in serum levels of allergen-specific IgG antibodies in AD patients. The serum levels of IL-10 and IFN-γ were significantly increased at 4 weeks after intramuscular administration of autologous

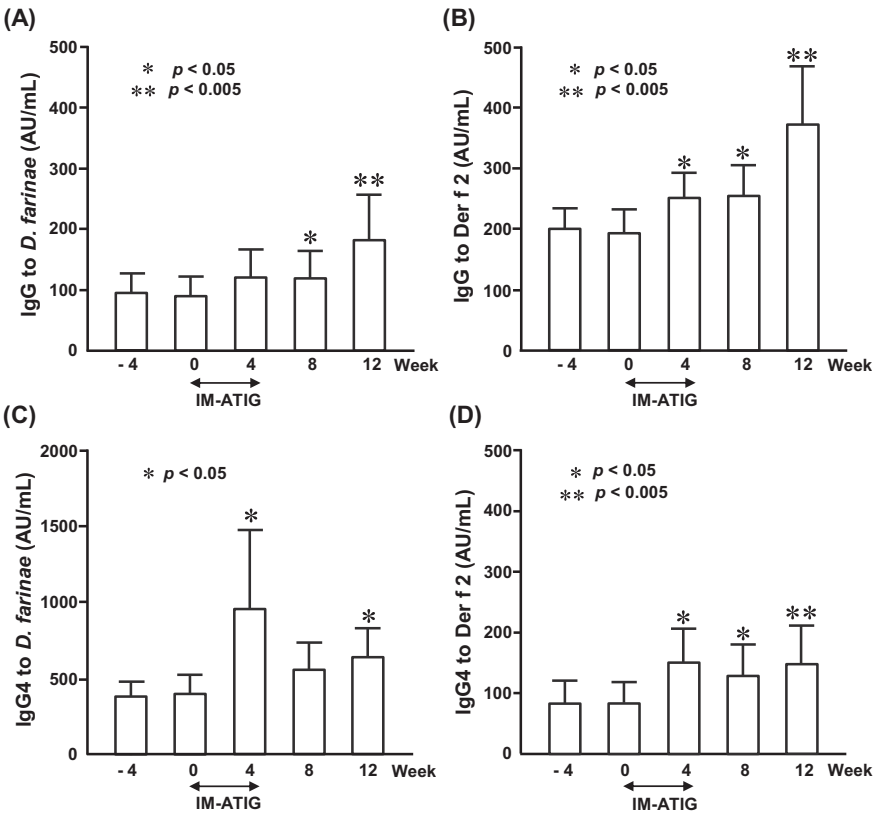


Fig. 3. Changes in serum levels of IgG and IgG4 antibodies to house dust mite allergens (whole body extract of *D. farinae* and recombinant Der f 2 allergen) in 16 adult patients with atopic dermatitis who received intramuscular administrations of 50 mg autologous total IgG (IM-ATIG) twice a week for 4 weeks. Data are means ± SEM. $^{*}p < 0.05$, $^{**}p < 0.005$ compared with baseline (Wilcoxon signed-rank test).

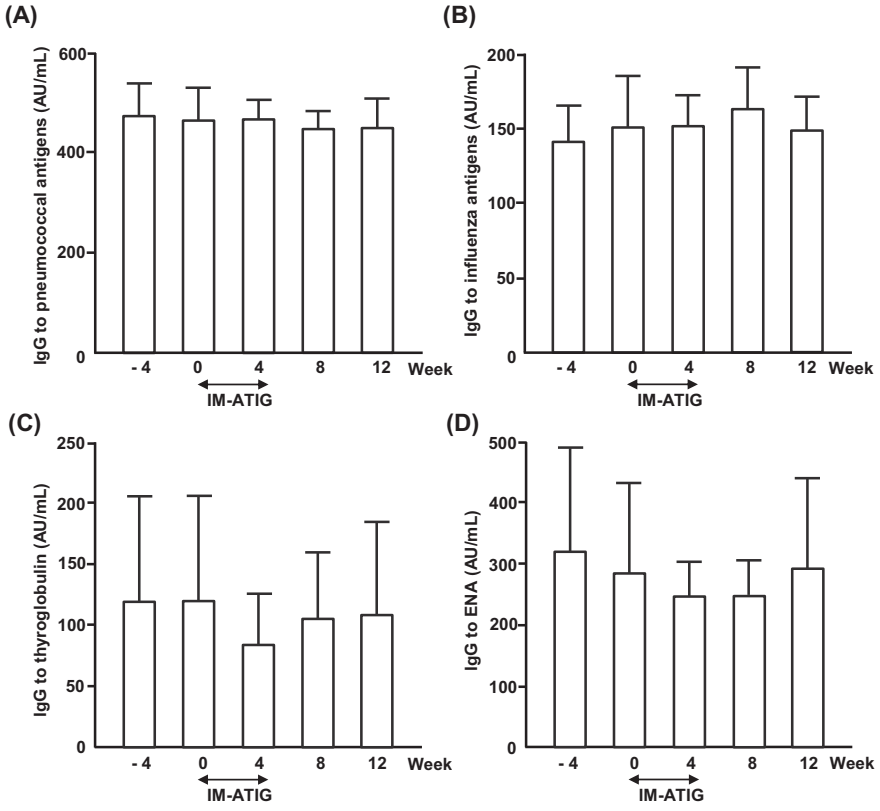


Fig. 4. Changes in serum levels of IgG antibodies to pneumococcal capsular polysaccharide antigens (A), influenza antigens (B), bovine thyroglobulin (C), and bovine thymus extract (as extractable nuclear antigens, ENA) (D) in 16 adult patients with atopic dermatitis who received intramuscular administrations of 50 mg autologous total IgG (IM-ATIG) twice a week for 4 weeks. Data are means ± SEM.

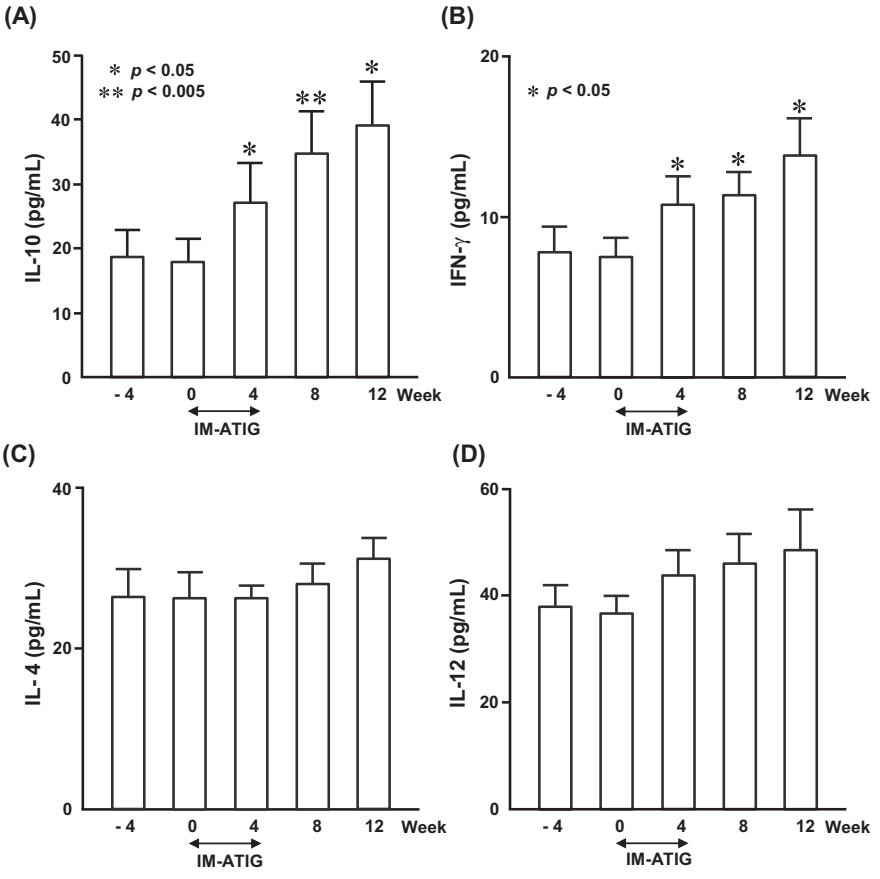


Fig. 5. Changes in serum levels of IL-10 (A), IFN-γ (B), IL-4 (C), and IL-12 (D) in 16 adult patients with atopic dermatitis who received intramuscular administrations of 50 mg autologous total IgG (IM-ATIG) twice a week for 4 weeks. Data are means ± SEM. *$p < 0.05$, **$p < 0.005$ compared with baseline (Wilcoxon signed-rank test).

total IgG compared with baseline in AD patients. These results suggest that intramuscular administration of autologous total IgG activated regulatory T cells secreting IL-10 and Th1 cells secreting IFN-γ in AD patients. We speculate that IL-10 and IFN-γ secreted from activated T cells suppressed the production of allergen-specific IgE antibodies and increased that of allergen-specific IgG antibodies by B cells in AD patients.

IgE antibodies to common inhalant allergens, including HDM and pollens, play a key role in the development of allergic diseases including allergic asthma, allergic rhinitis, and AD [19]. IgG antibodies to those allergens are also present in the blood of patients with allergic diseases [20]. Antigenic cross-reactivity between the idiotype of allergen-specific IgE antibodies and that of allergen-specific IgG antibodies has been reported in patients with allergic disease [21]. Administration of polyclonal allergen-specific IgG antibodies was effective for prevention and treatment of IgE-mediated allergy in a mouse model of ovalbumin allergy [22]. We speculate that intramuscular administration of autologous total IgG containing allergen-specific IgG antibodies downregulates IgE-mediated allergic reactions and induces clinical improvements in patients with AD by stimulating an anti-idiotype immune response to allergen-specific IgE antibodies as a result of cross-reactivity between the idiotype of allergen-specific IgG antibodies and that of allergen-specific IgE antibodies. However, we did not detect T-cell responses or anti-idiotype antibodies to allergen-specific IgE antibodies because of technical difficulties with purifying IgE (very low serum concentration of IgE) in this study. Further studies are necessary to evaluate the immunological mechanism underlying induction of anti-allergic immunomodulatory effects by intramuscular administration of autologous total IgG.

Interestingly, the patterns of the changes in serum allergen-specific antibody levels induced by intramuscular administration of autologous total IgG (decrease in the serum levels of allergen-specific IgE antibodies and increase in those of allergen-specific IgG antibodies) are similar to the patterns of the long-term changes in serum allergen-specific antibody levels induced by allergen immunotherapy in patients with allergic rhinitis or allergic asthma [23]. Allergen immunotherapy involves administering gradually increasing doses of allergens to patients with allergic diseases via the subcutaneous or sublingual route [24]. Allergen immunotherapy has been shown to decrease hypersensitivity to the allergen and eventually reduce clinical symptoms in patients with allergic asthma, allergic rhinitis, and AD [24]. However, the onset timing of decreases in serum allergen-specific IgE antibody levels induced by allergen immunotherapy in patients with allergic diseases (1–3 years) is markedly delayed compared with intramuscular administration of autologous total IgG in AD patients [23]. There were no significant differences in the serum levels of allergen-specific IgE antibodies at 1 or 2 years after allergen immunotherapy in AD patients with IgE-mediated sensitization to HDM compared with baseline in previous studies [25,26]. However, significant decreases in the serum levels of allergen-specific IgE antibodies were observed at 12 weeks after intramuscular administration of autologous total IgG in this study. These results suggest that the mechanisms underlying the immunomodulatory effects induced by these two approaches are different.

Theoretically, nonspecific immunosuppression or autoimmunity could develop after intramuscular administration of autologous total IgG. However, no significant systemic side effects, including infection and autoimmune disease, were observed after intramuscular administration of autologous total IgG in previous short-term and long-term clinical studies involving 20 adult AD patients [8–10]. In this study, there were no significant differences in the serum levels of IgG antibodies to infectious antigens or autoantigens before and after intramuscular administration of autologous total IgG in AD patients. These results provide additional evidence of the clinical safety of intramuscular administration of autologous total IgG in AD patients.

In conclusion, intramuscular administrations of autologous total IgG induced anti-allergic immunomodulatory effects in AD patients. Further studies are required to evaluate the immunological mechanism underlying these effects.

Acknowledgments

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Conflict of interest

The authors have no conflicts of interest to declare.

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