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PII: S1081-1206(22)00293-9
DOI: <https://doi.org/10.1016/j.anai.2022.03.031>
Reference: ANAI 3906



To appear in: *Annals of Allergy, Asthma Immunology*

Received date: 13 January 2022
Revised date: 10 March 2022
Accepted date: 16 March 2022

Please cite this article as: Huan Zhang MD , Mo Xian PhD , Xu Shi PhD , Tian Luo MD , Qiujuan Su MD , Jing Li MD , Mulin Feng MD , Blocking function of allergen-specific IgG, F(ab')₂, and Fab antibodies prepared from patients undergoing Dermatophagoides pteronyssinus immunotherapy, *Annals of Allergy, Asthma Immunology* (2022), doi: <https://doi.org/10.1016/j.anai.2022.03.031>

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Blocking function of allergen-specific IgG, F(ab')₂, and Fab antibodies prepared from patients undergoing *Dermatophagoides pteronyssinus* immunotherapy

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Conflict of Interest

The authors have no conflicts of interest to declare.

Funding Sources

This study was supported by the National Natural Science Foundation of China [81500024], High Level Scientific Research Project of People's Hospital of Yangjiang [G2020004] to Mulin Feng, and supported by the Breeding Program of Major Research Plan [91542104] of the National Natural Science Foundation of China to J. L.

Clinical Trial Registration

This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University and registered in the Chinese Clinical Trial Registry (ChiCTR-OOC-15006207, <https://www.chictr.org.cn/enindex.aspx>).

Keywords

asthma; rhinitis; allergen immunotherapy; immunoglobulin G; immunoglobulin E

Abbreviations

AIT: allergen immunotherapy

BATI: basophil activation inhibition assays

CRIA: cytokine release inhibition assay

Der-f: *Dermatophagoides farinae*

Der-p: *Dermatophagoides pteronyssinus*

FcεR I: high-affinity IgE receptors

FcεR II: low-affinity IgE receptors

FEV₁: forced expiratory volume in one second

HDM: house dust mite

IgE-FAB: IgE-facilitated allergen binding to B-cells

IL: interleukin

PBMC: peripheral blood mononuclear cell

SCIT: subcutaneous allergen immunotherapy

sIgE: specific immunoglobulin E

sIgG4: specific immunoglobulin G4

SPT: skin prick test

Th: T helper cell

TNF: tumor necrosis factor

tIgG: total immunoglobulins G

tIgG4: specific immunoglobulins G4

tIgE: total immunoglobulins G4

Abstract

Background: The blocking function of allergen-specific F(ab')₂ [sF(ab')₂] and Fab (sFab) fragment antibodies prepared from allergen immunotherapy (AIT)-induced specific immunoglobulins G (sIgG) has not been fully investigated.

Objective: We aimed to investigate the inhibitory function of sIgG, sF(ab')₂, and sFab antibodies in patients undergoing Dermatophagoides pteronyssinus (Der-p) subcutaneous immunotherapy (SCIT).

Methods: This study involved 10 subjects (aged 18-42 years) with house dust mite allergic rhinitis and/or asthma who received a 156-week course of Der-p SCIT. Total IgG were purified from the serum of participants at week 0 and 156 by protein-A affinity chromatography. Der-p sIgG were purified by affinity chromatography with Der-p as a ligand at week 156. The sF(ab')₂ and sFab antibodies were prepared from Der-p sIgG by treatment with pepsin and papain, respectively. Immunoglobulin E (IgE)-facilitated allergen binding assay, basophil activation inhibition test, and cytokine release inhibition assay were employed to assess the inhibitory function of Der-p sIgG, sF(ab')₂, and sFab antibodies.

Results: We found that the Der-p sIgG, sF(ab')₂, and sFab antibodies significantly blocked Der-p/allergen-specific IgE (sIgE) complex binding to B cells, inhibited basophil activation, and significantly reduced the production of interleukin (IL)-5, IL-13, IL-17, and tumor necrosis factor (TNF)- α by peripheral blood mononuclear cells.

Conclusion: SCIT-induced Der-p sIgG, sF(ab')₂, and sFab antibodies may block the formation of Der-p/sIgE complexes, and may serve as a potential allergen-targeted biologics candidate for the treatment of allergic asthma.

INTRODUCTION

House dust mite (HDM) is a major cause of allergic asthma in China. The allergen complexing with specific immunoglobulins E (sIgE) on sensitized cells is the critical pathway for allergic reaction. The best strategy for treating allergic diseases is a combination of approaches, including avoiding allergens, patient education, appropriate drug therapy, and allergen immunotherapy (AIT). The efficacy of AIT has been confirmed for the treatment of allergic rhinitis, allergic asthma, and other IgE-mediated allergic diseases¹. Mechanisms of AIT involve a series of immunological changes, including generation of allergen-specific T regulatory and suppression of allergen-specific T helper (Th) 2 cells at the early stage, and the induction of interleukin (IL)-10^{2,3}. IL-10 can indirectly inhibit IgE production and directly drive the production of immunoglobulins G (IgG)⁴. Our previous and other studies have reported an increase of 10- to 100-fold in allergen-specific IgG4 (sIgG4) concentrations during AIT^{5,6}. Specific IgG4 antibodies are responsible for capturing the allergens before they reach IgE and reducing allergen density on the surface of IgE receptor-expressing effector cells, including mast cells and basophils, thereby preventing the activation of these cells⁷. However, quantitative assessments of IgG4 antibodies have shown a poor correlation with clinical response, as measured by symptom and medication scores^{8,9}. One possible explanation for this discrepancy is that AIT increases the serum level of IgG4 antibodies and alters their binding specificity and/or affinity^{10,11}. Thus, a functional, rather than just quantitative, assessment of IgG4 was proposed to evaluate their immunologic efficacy. Additionally, AIT is a typical immunotherapy model that induces B cell to produce sIgG. Indeed, using the immunized PBMCs may facilitate the implementation of genetic technology to generate fully human allergen-specific F(ab')₂ [sF(ab')₂] or Fab (sFab) antibodies *in vitro* by using the immunized PBMCs. These antibodies, constituted uniquely of human amino acid sequences and therefore with low

immunogenicity, may avoid the induction of neutralizing antibodies that could diminish their efficacy, as demonstrated by the efficacy and safety of fully human IgG4-based monoclonal antibody against IL-4 receptor α used in treatment for uncontrolled severe asthma¹². Thus, the construction of fully human allergen-specific antibodies may be helpful for the treatment and prevention of allergic diseases.

In this study, we purified the total IgG from the serum of *Dermatophagoides pteronyssinus* (Der-p) subcutaneous allergen immunotherapy (SCIT) patients by protein-A affinity chromatography. In addition, the allergen sIgGs were purified by Der-p-liganded affinity chromatography. The Der-p sIgG was treated with pepsin and papain to prepare the sF(ab')₂ and sFab antibodies. The blocking function of sIgG, sF(ab')₂, and sFab antibodies was assessed and compared to evaluate the potential application of allergen-targeted antibodies on the prevention of allergic diseases.

METHODS

Study design and population

The study included ten subjects (6 males and 4 females, 18–42 years of age) who fulfilled the criteria of the International Allergic Rhinitis and its Impact on Asthma (ARIA) guideline and/or the Global Initiative for Asthma (GINA) guideline for mild-to-moderate asthma^{13, 14}, and had positive skin prick test (SPT) and sIgE to Der-p. Ten healthy subjects were taken as the baseline control (Table 1). All patients came from the Allergy and Clinical Immunology Department of Guangzhou Institute of Respiratory Diseases and received Der-p SCIT. Blood samples were collected before treatment (week 0) and at week 156 when the patients visited the hospitals for treatments. The study flowchart is shown in Figure 1. This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University and registered in the Chinese Clinical Trial Registry (ChiCTR-OOC-15006207). Written informed consent was obtained from all patients.

Skin prick tests

Sensitization to eight common aeroallergens was assessed by SPT with allergen extracts (Soluprick SQ, ALKAbello A/S, Horsholm, Denmark). These extracts included Der-p, *Dermatophagoides farinae* (Der-f), *Blomia tropicalis*, cat dander, dog dander, cockroach, ragweed, and mugwort pollen. A positive SPT was defined as a wheal diameter ≥ 3 mm, after subtraction of the physiological saline control.

Detection of serum IgE and IgG4

Pharmacia CAP fluorescence enzyme-linked immunoassay (ELISA) system (ThermoFisher, Sweden) was employed to measure the levels of total IgE (tIgE) and

sIgE against Der-p, Der-f, and other common allergens in all serum samples. The sIgE results, reported as kU/l, are in the range of 0.35 to 100 kU/l. Any sample with sIgE level > 100 kU/l was diluted and retested. The levels of serum Der-p sIgG4 were measured by a four-layer sandwich ELISA as we reported previously, and the IgG4 concentration was expressed in arbitrary units (AU/mL)¹⁵.

SCIT protocol

The SCIT-treated patients received aluminum-formulated Der-p Alutard-SQ vaccine (ALK-Abello A/S) according to a protocol we described previously¹⁵. SCIT was administered in the clinic and included a 16-week recommended dosing schedule (weekly subcutaneous injections) followed by a maintenance dose of 100,000 Alutard-SQ (given every 6 weeks) until completion of a three-year treatment. Throughout the study, medications such as oral antihistamines and intranasal steroids, were provided to all patients as needed to relieve rhinitis and asthma symptoms.

Purification of allergen-specific IgG antibodies

For purification of total IgG, the serum samples from SCIT patients at week 0 and week 156 (2 mL, diluted 1:10 in binding buffer) were loaded into affinity chromatography columns (Bio-Scale Mini UNOsphere SUPRA Protein-A cartridges, Bio-rad, USA), previously equilibrated with binding buffer (0.02 M sodium phosphate, 0.02 M sodium citrate, pH 7.5). Samples were washed with 10 volumes of binding buffer. Total IgG was then eluted in the elution buffer (0.02 M sodium citrate, 0.1 M sodium chloride, pH 2.5). Three milliliter fractions were collected, and the pH was neutralized with 1 M Tris-HCl (pH 9.0). For further purification of Der-p sIgG, affinity chromatography with Der-p 1 (INDOOR Biotechnologies, USA) as a ligand was used. In brief, Der-p 1 (10 mg/mL) was coupled to HiTrap NHS-activated HP

columns (GE Healthcare, UK) and equilibrated with binding buffer (0.02 M sodium phosphate, 0.02 M sodium citrate, pH 7.5). Total IgG from SCIT patients at week 156 were applied to the Der-p 1 coupled columns. Samples were washed with binding buffer and eluted with elution buffer (0.02 M sodium citrate, 0.1 M sodium chloride, pH 2.5). Purified total IgG and sIgG were concentrated using an ultrafiltration centrifuge tube (Millipore, USA), quantitated with bicinchoninic acid (BCA) protein assay (Pierce BCA Protein Assay Kit, Thermo Scientific, USA) and diluted to a final concentration of 2 mg/mL.

Preparation of sF(ab')₂ and sFab fragments

Der-p sF(ab')₂ and sFab antibodies were obtained by pepsin and papain (Pierce Preparation Kit, Thermo Scientific, USA) digestion, respectively, according to the manufacturer's instructions. Briefly, 0.5 mL of prepared total IgG (week 0 and week 156) and Der-p sIgG (week 156) were added to the spin column tube containing the equilibrated immobilized papain or pepsin. The digestion reaction was incubated for 3 hours with an end-over-end mixer at 37°C to generate Fab and F(ab')₂ antibodies. The generated antibodies were applied to the protein-A spin column to remove the undigested IgG. Purified F(ab')₂ and Fab from total IgG week 0 [tF(ab')₂-0w and tFab-0w], F(ab')₂ and Fab from total IgG week 156 [tF(ab')₂-156w and tFab-156w], and sFab and sF(ab')₂ from Der-p sIgG antibodies were concentrated with an ultrafiltration centrifuge tube, measured with BCA, and adjusted to a final concentration of 2 mg/mL.

IgE facilitated allergen binding assay

Serum inhibitory activity against allergen/sIgE binding to B cells (IgE-FAB) was tested as described by Shamji et al. and our previous study^{15, 16}. Briefly, stock

indicator serum containing a high concentration of Der-p (192 kU/l) sIgE was preincubated with Der-p and test samples [tIgG-0w, tF(ab')₂-0w, tFab-0w, tIgG-156w, tF(ab')₂-156w, tFab-156w sIgG, sF(ab')₂, and sFab] at 37°C for 1 h to form allergen/IgE complexes. EBV-transformed B cells (a gift from ALK-Abelló A/S) with high expression of low-affinity IgE receptor (FcεR II, also called CD23) at 10⁵ cells per sample were then added to the allergen/IgE mixture and incubated at 4°C for 1 h. Cells were washed, stained with anti-IgE-FITC antibodies (AbD Serotec, UK), and detected by flow cytometry.

Basophil activation and cytokine release inhibition assays

Basophil activation inhibition assays (BATI) were performed, as we reported previously⁵. Cytokine release inhibition assays (CRIA) were also performed in this experiment. Briefly, tIgG-0w, tF(ab')₂-0w, tFab-0w, tIgG-156w, tF(ab')₂-156w, tFab-156w sIgG, sF(ab')₂, and sFab antibodies (20 µL at 2 mg/mL) were incubated with 60 µL of Der-p (final concentration of Der-p was 0.15 µg/mL) at 37°C for 1 h. A 200 µL (2 × 10⁶ cells/mL) of peripheral blood mononuclear cells (PBMCs) from HDM atopic adult volunteers were then added to the antibody/allergen mixture and incubated for 30 min at 37°C. After centrifugation, the supernatants were collected and stored at -20°C for cytokine measurement. Cells were stained with CD123-PE-Cy5 (BD, USA), HLA-DR-APC (BD), CD203c-PE (Beckman Coulter, USA), and CD63-FITC (Beckman Coulter). Basophils gated as SSC^{low}/CD203c⁺/CD123⁺/HLA-DR⁻ were detected by flow cytometry (BD FACSVerse, USA) and analyzed using FCS Express software version 4 (De Novo Software, Pasadena, USA). The supernatant concentration of IL-5, IL-13, IL-17, and tumor necrosis factor (TNF)-α were measured by Bio-Plex Cytokine Assay (Bio-rad, USA) according to the accompanied product instruction.

Statistical analysis

Statistical analysis was performed using SPSS (version 16.0, SPSS Inc., Chicago, IL, USA) and GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, USA).

Independent-sample t-test and paired-sample t-test were used to analyze between-group differences and within-group differences of the baseline data. In addition, one-way ANOVA was used to analyze the difference of IgE-FAB, BATI, and CRIA in inhibition antibodies. Values are shown as mean $\pm$ SEM. Differences were considered significant at P values < 0.05 .

RESULTS

Patient characteristics

Baseline (week 0) demographic data, lung function, antibody levels, and levels of cytokines in the supernatant PBMCs cultures of the SCIT and healthy participants are shown in Table 1. There were no differences between the SCIT and healthy groups in terms of sex, age, and forced expiratory volume per second, shown as percent predicted ($FEV_1\%$) ($P > 0.05$). However, Der-p and Der-f SPT-diameter, sIgE, and total IgE levels were higher in the SCIT subjects than in the healthy controls at baseline ($P < 0.001$). In addition, there was a significant increase of IL-5, IL-13, IL-17, and TNF- α secretion by the PBMCs from the SCIT subjects upon Der-p stimulation ($P < 0.001$).

Serum sIgG4 and preparation of IgG, Fab, and F(ab')₂ antibodies

Levels of Der-p sIgG4 in SCIT patients significantly increased after 156 weeks of SCIT ($P < 0.001$) (Figure 2A). The preparation of IgG, Fab, and F(ab')₂ fragments was verified by non-reduced SDS-PAGE (example on Figure 2B). Silver staining indicated that the whole serum (lane a), filtered solution through Protein-A cartridge (lane b), and sIgG antibody had molecular weights of approximately 150 kDa (lane c). The sF(ab')₂ antibody migrated with a molecular weight of 100 kDa (lane d), sFab antibody at 45 kDa, and Fc fragments at 40 kDa (lane e).

Blocking of allergen-IgE complex binding to Fc ϵ R II

The IgE-FAB result revealed that tIgG-156w, sIgG, tF(ab')₂-156w, sF(ab')₂, tFab-156w, and sFab antibodies obtained from SCIT patients at week 156 had increased capacity to inhibit Der-p-allergen sIgE complex from binding to Fc ϵ R II

compared with the antibodies obtained at week 0 ($P < 0.01$, Figure 3A–C). The sIgG, sF(ab')₂, and sFab antibodies showed a higher inhibitory capacity compared with tIgG-156w, tF(ab')₂-156w, and tFab-156w antibodies ($P < 0.05$, Figure 3A–C).

Inhibition of basophil activation and cytokine release

BATI assays showed that tIgG-156w, sIgG, tF(ab')₂-156w, sF(ab')₂, tFab-156w, and sFab antibodies obtained from SCIT patients at week 156 had a significantly increased capacity to inhibit basophil activation upon challenge with Der-p relative to the antibodies obtained at week 0 ($P < 0.001$, Figure 3D–F). The sIgG, sF(ab')₂, and sFab antibodies exerted a higher blockade of basophil activation compared with tIgG-156w, tF(ab')₂-156w, and tFab-156w antibodies ($P < 0.001$, Figure 3D–F). The CRIA demonstrated that the tIgG-156w, sIgG, tF(ab')₂-156w, sF(ab')₂, tFab-156w, and sFab antibodies obtained from SCIT patients at week 156 significantly inhibited the IL-5, IL-13, IL-17, and TNF- α release by Der-p stimulated PBMCs ($P < 0.01$, Figure 4A–L). The sIgG, sF(ab')₂, and sFab antibodies showed enhanced blocking capacity of Der-p stimulated PBMC cytokine release compared with tIgG-156w, tF(ab')₂-156w, and tFab-156w antibodies ($P < 0.01$, Figure 4A–L).

DISCUSSION

In this study, we found that AIT could induce a substantial increase in Der-p sIgG4. We also demonstrated that Der-p sIgG, sF(ab')₂, and sFab antibodies prepared from AIT patients could inhibit Der-p-sIgE complex binding to FcεR II and block the activation of basophils and cytokine release by effector Th2 cells.

Our findings and those of other studies confirmed that sIgG4 induction was a prominent immunological change induced by AIT^{15, 17, 18}. The ratio of IgG4 antibodies to total IgG antibodies was up to 75% after AIT, yet this ratio accounted for only 4% in healthy individuals^{19, 20}. The allergen concentration and treatment period may affect the magnitude of sIgG4 concentration during AIT^{21, 22}. Besides quantitative assessments of IgG4 antibodies, several assays have been established to measure the functional activities of IgG in blocking allergen/IgE complexes binding to high-affinity IgE receptors (FcεR I) and FcεRII cells, including IgE-facilitated allergen presentation (IgE-FAP) to T cells¹¹, IgE-FAB¹⁶, and BATI⁵.

In the current study, we found that sIgG antibodies could inhibit Der-p/sIgE complex binding to FcεR II-expressing cells, which is in agreement with our previous study¹⁵ and other studies^{23, 24}. Our results also showed that sF(ab')₂ and sFab antibodies could compete with sIgE to form an allergen-IgE complex, thereby inhibiting the complex binding to B cells and preventing allergen-dependent T cell activation⁹. FcεRII is a low-affinity IgE receptor and a negative regulator of IgE synthesis^{25, 26}. A previous study indicated that Der-p 1 exerted a proteolytic activity resulting in FcεRII cleavage²⁷. Therefore, cleavage of FcεRII by Der-p 1 from the surface of B cells would potentially disrupt the negative feedback signal, causing excessive IgE synthesis²⁷ and favoring a Th2 type response²⁸. Thus, allergen sIgG, sF(ab')₂, and sFab antibodies may reduce the production of IgE antibodies by forming sIgG/sF(ab')₂/sFab-allergen complexes and inhibiting Der-p 1 cleavage of FcεRII in susceptible atopic individuals.

Basophils and mast cells are primary effectors responsible for the release of inflammatory mediators. However, there is a lack of data regarding the effect of inhibitory antibodies on those effector cells in the investigation of AIT mechanism [44]. Our previous study and other studies found that allergen-specific IgG could operate by reducing basophils' threshold sensitivity at submaximal Der-p allergen concentration rather than basophils' reactivity^{5, 29}. This study also demonstrated that sF(ab')₂ and sFab antibodies inhibited Der-p allergen from activating the basophils. Basophils typically express a high level of FcεR I. Therefore, blocking antibodies may inhibit other FcεRI-expressing effector cells, such as mast cells, monocytes, platelets, eosinophils, dendritic, and Langerhans cells. IgG4 is a unique antibody isotype involved in a continuous process of heavy and attached light-chain exchange, referred to as "Fab-arm exchange"²⁰. These characteristics of sIgG4 result in monovalent and non-crosslinking antibodies. The magnitude of effector cell responses involves the expression level of FcεRI on the cell surface³⁰. Thus, the binding of IgG4 and allergens could reduce the concentration of the free allergen able to bind IgE without inducing effector cell activation. The factors related to inhibition of immunological reactions triggered by allergens may involve interactions between effector cells, IgE, and the specificity and affinity of different IgG subclasses³¹. In addition, the affinity of different IgG subtypes such as IgG1, IgG2, and IgG4 might contribute to differential inhibitory associations. Additional studies involving purified allergen-specific IgGs of different subclasses, IgE, and effector cells are needed to clarify the interrelationship between these cellular responses and humoral allergen-specific IgG and IgE antibodies.

This study showed that the baseline level of Th2 cytokines (IL-5, IL-13, IL-17, and TNF-α) in allergic patients increased significantly after Der-p stimulation. These data confirmed that the cross-linking of IgE bound to the surface of effector cells could trigger a series of signal transductions and cause the cells to degranulate and release inflammatory mediators³². Recent studies suggested that biological therapies

targeting inflammatory mediator release could be a potential treatment for this allergic airway disease based on the knowledge of its pathobiology³³. A humanized monoclonal IgE-targeting antibody, Omalizumab, provides a long-term control in moderate-to-severe allergic asthma³⁴. Monoclonal antibodies targeting IL-5 or its receptor (IL-5R) have been proven effective against eosinophilic asthma refractory to existing treatments³⁵. Antibodies targeting IL-4, IL-13, IL-6, and IL-23 have also been under investigation³³. Additionally, there are some investigations on antibodies targeting the allergens, which are the initiators of allergies by triggering an immediate-type immune reaction and Th2 cytokine release in allergic patients^{36, 37}. Passive immunization with allergen-specific IgG antibodies in combination with active AIT has been used to reduce adverse reactions during AIT³⁸⁻⁴⁰. Passive immunization with specific IgG antibodies in experimental animals could also suppress allergic sensitization, reduce allergic inflammation, and prevent allergy development^{37, 41-43}. Moreover, AIT-induced allergen-specific IgG antibodies could cross the placenta⁴⁴ and may induce tolerance to allergens in the offspring⁴⁵. In this study, we found that not only sIgG but also sF(ab')₂ and sFab fragment antibodies could compete with sIgE binding to allergen and inhibit cytokine release from effector cells. Generating fully human allergen-specific Fab or F(ab')₂ antibodies *in vitro* by genetic technology using the immunized PBMCs may avoid the induction of neutralizing antibodies, and may be helpful for the treatment and prevention of allergic diseases.

The novelty of our study is that we demonstrated the allergen-specific F(ab')₂ and Fab antibodies could inhibit the formation of Der-p/sIgE complexes and cytokine release by activated effector cells. There are several limitations, however. First, the affinity of the sIgG, sF(ab')₂, and sFab antibodies was not assessed. Second, the sF(ab')₂ and sFab were not purified and contained Fc or other fragments, and those fragments could have affected the concentration of antibodies and the results. Finally, additional studies with purified sF(ab')₂ and sFab antibodies are needed to confirm the

function of sIgG, sF(ab')₂, and sFab antibodies on the treatment and prevention of allergy in an experimental animal model.

In conclusion, we demonstrated that AIT-induced allergen sIgG, sF(ab')₂, and sFab antibodies have blocking function against sIgE activity, reducing basophil sensitivity to the allergen and cytokine release by effector cells. Thus, the construction of allergen-specific inhibition antibodies may have potential applications for treatment and prevention of allergic diseases.

Acknowledgments

We thank LetPub (www.letpub.com) for linguistic assistance and pre-submission expert review.

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Figure Legend

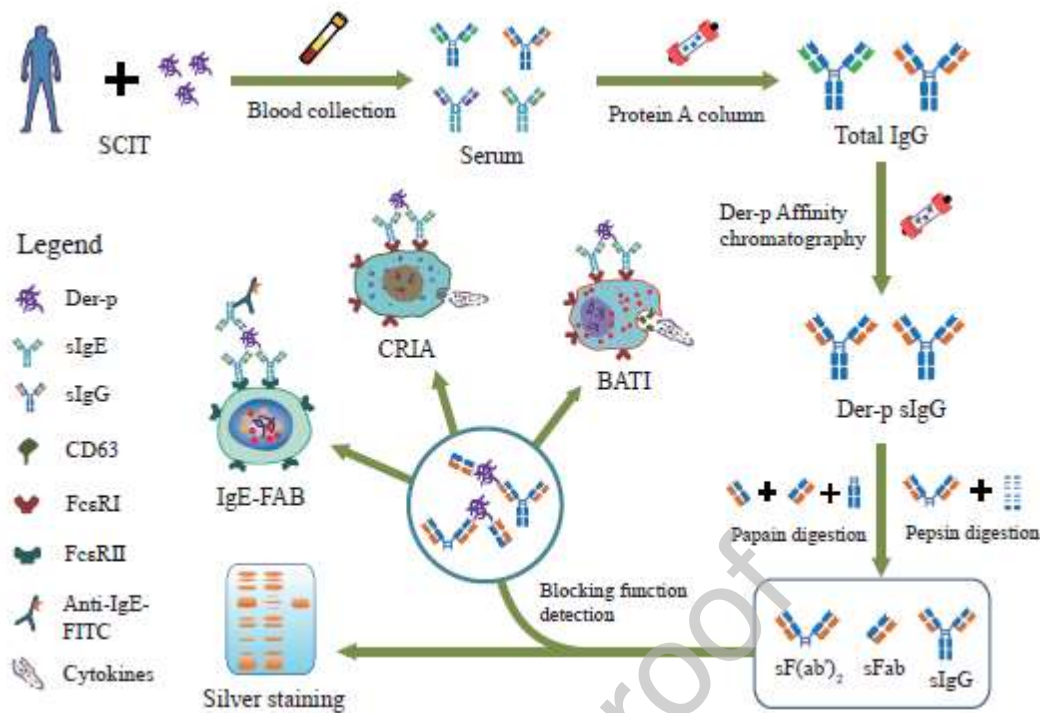


Figure 1. Flowchart of the study design. Procedure for purification of the total IgG antibodies from serum samples by affinity chromatography and the preparation and functional investigation of the sF(ab')₂ and sFab antibodies. SCIT, subcutaneous allergen immunotherapy; IgG, immunoglobulins G; Der-p, *Dermatophagoides pteronyssinus*; sIgG: specific immunoglobulins G; IgE-FAB, IgE-facilitated allergen binding to B-cells; CRIA, cytokine release inhibition assay; BATI, Basophil activation inhibition test; sIgE: specific immunoglobulin E; FcεR I, high-affinity IgE receptors; FcεR II, low-affinity IgE receptors; FITC, fluorescein isothiocyanate.

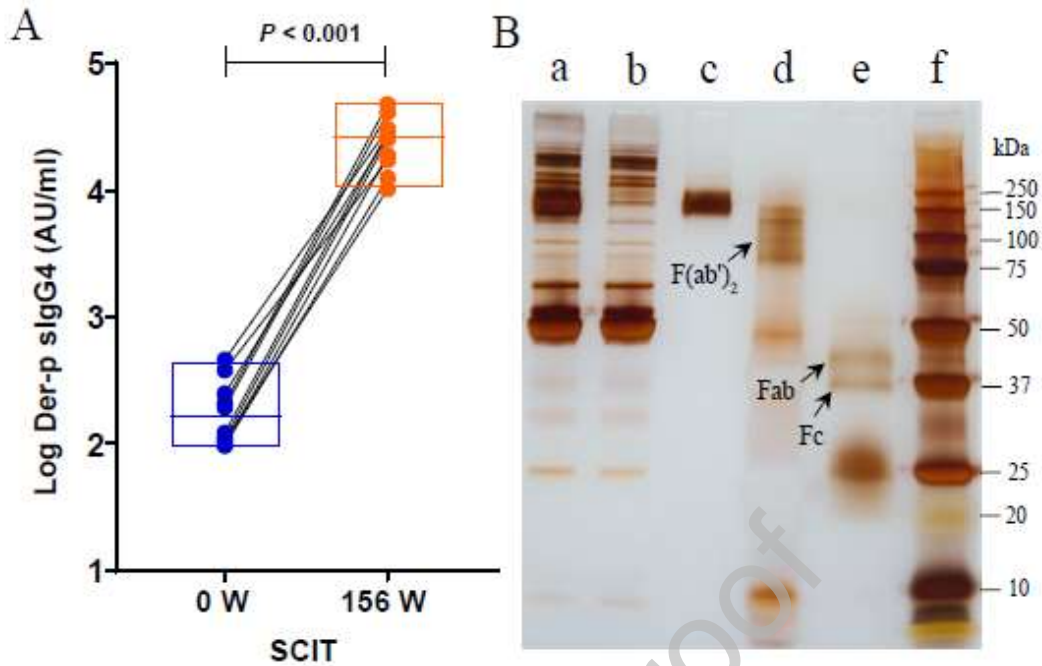


Figure 2. sIgG4 induction in SCIT patients and sIgG, sF(ab')₂ and sFab preparation. Change in Der-p sIgG4 concentration assessed by ELISA (A), and purification of sIgG, sF(ab')₂ and sFab antibodies monitored by non-reduced SDS-PAGE (B). The whole serum (lane a), the filtered solution through Protein-A cartridge (lane b), the sIgG antibody (lane c), the sF(ab')₂ antibody (lane d), the sFab antibody, and Fc fragments (lane e) are shown. Molecular weight markers are shown in kilodaltons (kDa) (lane f). SCIT, subcutaneous allergen immunotherapy; Der-p, *Dermatophagoides pteronyssinus*; sIgG4, specific immunoglobulin G4.

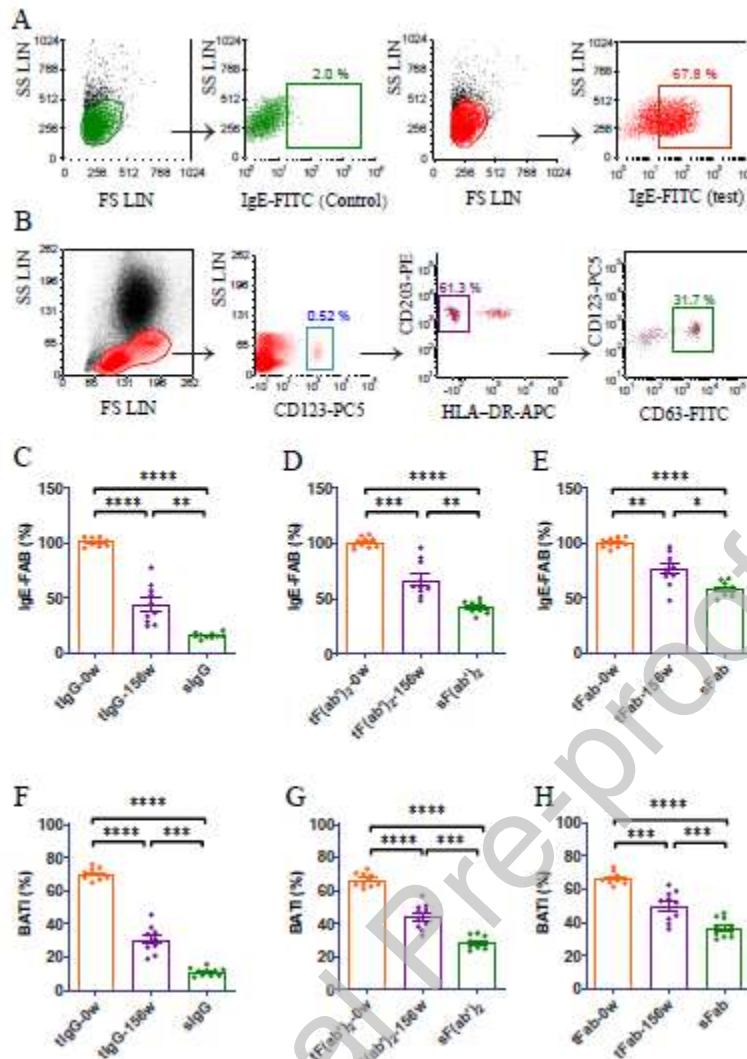


Figure 3. Assessment of the inhibitory function of the antibody preparations by IgE-FAB and BATI assays. The lymphocytes were identified by their scatter properties, the B cells carrying Der-p/sIgE complexes were gated as IgE-FITC⁺; sIgE without adding allergen was served as the negative control (A); the activated basophils were gated as SSC^{low}/CD203c⁺/CD123⁺/HLA-DR⁻ (B). The IgE-FAB and BATI results for the tIgG-0w, tIgG-156w, sIgG, tF(ab')₂-0w, tF(ab')₂-156w, sF(ab')₂, tFab-0w, tFab-156w, and sFab antibodies (C–H). **P* < 0.05, ***P* < 0.01, ****P* <

0.001, **** $P < 0.0001$. IgE-FAB, IgE-facilitated allergen binding to B-cells; BATI, Basophil activation inhibition assays.

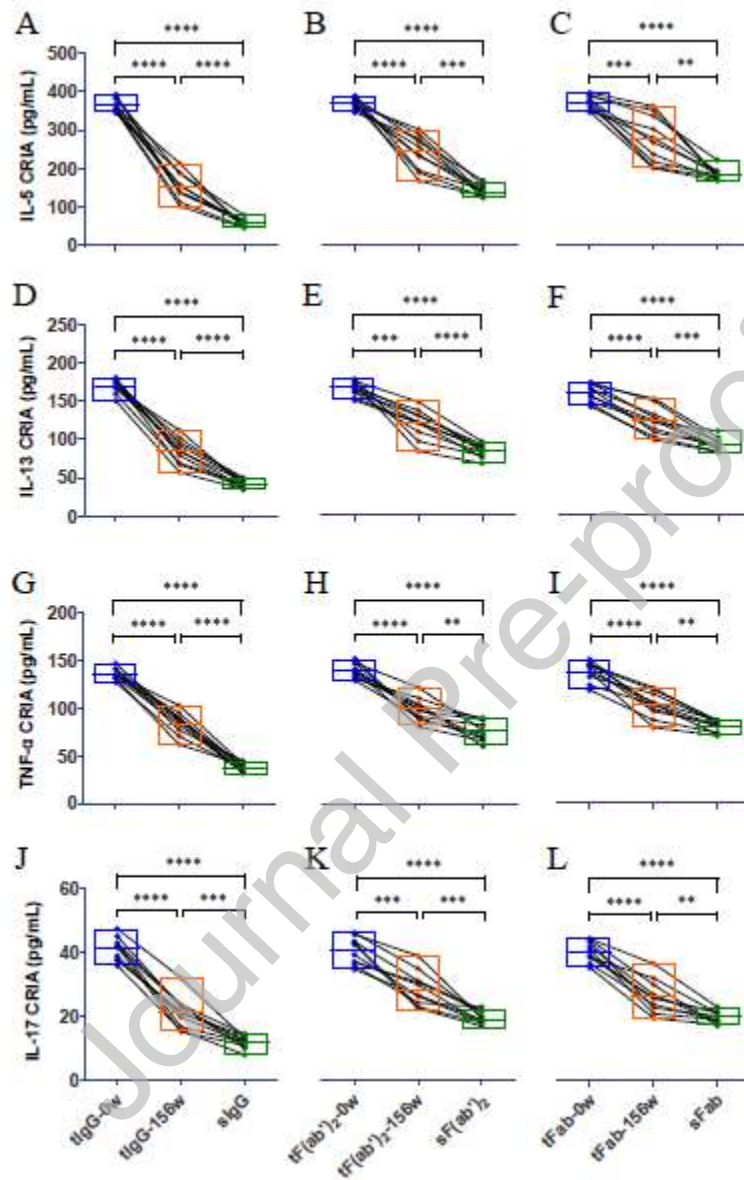


Figure 4. Inhibitory function of the antibody preparations in CRIA. CRIA results for the tIgG-0w, tIgG-156w, sIgG, tF(ab')₂-0w, tF(ab')₂-156w, sF(ab')₂, tFab-0w,

tFab-156w, and sFab antibodies (A–L). ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

CRIA, cytokine release inhibition assay; IL, interleukin; TNF, tumor necrosis factor.

Table 1. The baseline (week 0) demographic data, lung function, antibody levels, and levels of supernatant cytokines in PBMCs from the SCIT and healthy participants.

Variable	SCIT group		Healthy group	
Participants, <i>n</i>	10		10	
Sex (male/female), <i>n</i>	6/4		5/5	
Age distribution (years)	29.6 ± 2.7		23.8 ± 2.3	
SPT-diameter				
Der-p (mm)	9.5 ± 0.6		0.9 ± 0.0 †††	
Der-f (mm)	9.1 ± 0.5		0.9 ± 0.0 †††	
sIgE to Der-p (kU/L)	92.7 ± 9.5		0.4 ± 0.0 †††	
sIgE to Der-f (kU/L)	87.5 ± 9.3		0.3 ± 0.0 †††	
Total IgE (kU/L)	691.6 ± 36.4		51.8 ± 7.2 †††	
FEV ₁ (% predicted)	91.2 ± 1.6		94.8 ± 1.7	
Der-p (25 µg/mL)	Unstimulated	Stimulated	Unstimulated	Stimulated
Cytokines (pg/mL)				
IL-5	14.8 ± 4.2	1105.9 ± 162.4 ***	13.3 ± 3.3	19.2 ± 4.1 †††
IL-13	31.2 ± 8.4	576.2 ± 60.2 ***	28.9 ± 8.4	26.5 ± 9.8 †††

IL-17	12.3 ± 2.2	86.1 ± 13.2 ^{***}	11.0 ± 2.7	14.8 ± 3.6 ^{†††}
TNF- α	29.7 ± 8.7	308.7 ± 37.7 ^{***}	27.8 ± 7.3	30.7 ± 9.2 ^{†††}

Data are presented as mean ± SEM. ††† $P < 0.001$ when compared with SCIT group;

*** $P < 0.001$ when compared with unstimulated. PBMC, peripheral blood

mononuclear cell; SCIT, subcutaneous allergen immunotherapy; SPT, skin prick test;

IgE, immunoglobulin E; sIgE, specific IgE; FEV₁, forced expiratory volume in one

second; Der-p, *Dermatophagoides pteronyssinus*; Der-f, *Dermatophagoides farina*;

IL, interleukin; TNF- α , tumor necrosis factor- α .