

Prevention and treatment of allergic inflammation by an Fc γ -Der f2 fusion protein in a murine model of dust mite-induced asthma

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Abstract The immunoglobulin E (IgE) high-affinity receptor Fc ϵ RI expressed on mast cells and basophils plays a critical role in triggering allergic disease. The co-aggregation of the Fc ϵ RI and Fc γ RIIb receptors is inhibitory to Fc ϵ RI signaling and holds great potential for the treatment of IgE-mediated allergies. In China, *Dermatophagoides farinae* is a common anaphylaxis trigger. Therefore, in this study, the Fc γ RIIb-mediated immunomodulating activity of recombinant Fc γ -Der f2 fusion protein was tested in a Der f2-allergic murine model. Following the treatment, bronchoalveolar lavage fluid (BALF) was collected to measure the expression of several Th1/Th2-type cytokines (IL-5, TNF- α , IL-12p70, IL-4, IL-10, IFN- γ and IL-18) and histamine, while blood was used to detect the specific IgE and IgG-types anti-Der f2 antibodies, for measurement. In contrast to the saline-treated allergic mice, the levels of Der f2-specific IgE, cytokines and histamine were lowered in the Fc γ -Der f2-treated allergic mice, in addition to the rare inflammatory cell infiltration in the airways and blood

vessels revealed by histopathological examination. The recombinant Fc γ -Der f2 protein was demonstrated to function as an effective immunotherapeutic agent, suggesting that chimeric human Fc γ -allergen proteins could be used in the development of antigen-specific immunotherapy for human allergic diseases.

Keywords Fc ϵ RI · Fc γ RIIb · Fc γ -Der f2 fusion protein · Immunomodulation · Allergic asthma mouse

Abbreviations

BALF	The bronchoalveolar lavage fluid
IgE	Immunoglobulin E
IgG	Immunoglobulin G
ITAM	Immunoreceptor tyrosine-based activation motif
ITIM	Immunoreceptor tyrosine-based inhibitory motif

Introduction

Anaphylaxis is a serious and potentially life-threatening allergic reaction with a rapid onset. Despite this, anaphylaxis is not uncommon, and its incidence is increasing, especially in the developing countries including China. Current research efforts toward the management of acute anaphylaxis are focused on immunomodulation [1, 2], for example, by balancing the Th1/Th2 immune response, and on minimizing side effects. Pathologically, antigens induce anaphylaxis by causing IgE cross-linking and Fc ϵ RI aggregation on mast cells and basophils. Multivalent antigens cross-link Fc ϵ RI, leading to tyrosine phosphorylation of immunoreceptor tyrosine-based activation motif (ITAM) in the cytoplasmic tails of the β - and γ -Fc ϵ RI subunits, thereby triggering downstream signaling through Syk [3,

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4]. Mast cells and basophils also express Fc γ RIIb, which is a low-affinity IgG receptor that contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) within its cytoplasmic tail. Daeron et al. [5] reported that Fc ϵ RI activation in mast cells can be downregulated by inhibitory Fc γ Rs, suggesting that inhibitory Fc γ Rs may have therapeutic potentials. Indeed, Zhu et al. [6] reported that an antigen-nonspecific human bifunctional Fc γ -Fc ϵ fusion protein (GE2) caused co-aggregation of Fc ϵ RI with Fc γ RIIb and inhibited human basophil and mast cell functions in vitro and in vivo. Moreover, another form of immunotherapy based on a chimeric fusion protein composed of human Fc γ and the cat allergen Fel d1 (GFD) has been reported [7]. Although numerous potential anaphylaxis triggers exist, the most common inhaled trigger in China is *Dermatophagoides farinae* [8]. Therefore, we exploited the therapeutic value of a novel chimeric fusion protein containing the human IgG Fc γ and Der f2, which is a major allergen produced by *D. farinae*. We hypothesized that this chimeric Fc γ -Der f2 fusion protein might provide a new immunotherapy platform for treating antigen-specific allergies. In the present study, the Der f2 gene vector was cloned, and a recombinant Der f2 protein was obtained. We inserted the Der f2 gene downstream from IgG Fc γ in a eukaryotic expression vector. The recombinant Fc γ -Der f2 fusion protein was successfully expressed in HEK293 cells. The role of receptor-mediated immunomodulation was analyzed in a murine model of antigen-induced lung allergic inflammation elicited by intraperitoneal sensitization with Der f2 and treated with the Fc γ -Der f2 fusion protein. Our results indicate that the Fc γ -Der f2 fusion protein could be useful for the treatment of Der f2-induced allergy.

Materials and methods

Mice, reagents and antibodies

BALB/c female mice (6–8 weeks old) were obtained from Sion–British Sippr/BK Laboratory and housed in the Animal Experimental Center of Shanghai First People's Hospital under specific pathogen-free conditions. The Chancellor's Animal Research Committee approved all of the animal studies and confirmed that the experiments involving animals adhered to the guidelines set forth by the Shanghai Jiao Tong University School of Medicine.

The restriction enzymes BamH I, Xho I and Hind III were purchased from TaKaRa (Japan). Isopropyl β -D-1-thiogalactopyranoside (IPTG) was purchased from Sigma-Aldrich (St Louis, MO, USA). The mouse anti-6 $\times$ His tag antibody was purchased from MBL. Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG and HRP-conjugated goat

anti-human IgG (γ chain-specific) antibodies were purchased from Affinity BioReagents (Lake Placid, NY, USA). The HRP-conjugated goat anti-human IgE antibody was obtained from Invitrogen (Carlsbad, CA, USA), and the HRP-conjugated goat anti-mouse IgE monoclonal antibody was obtained from SouthernBiotech (Birmingham, AL, USA). Red blood cell lysis buffer was purchased from Imgenex (San Diego, CA, USA). The anti-human IgG affinity column was purchased from Pierce (Rockford, IL, USA).

Cloning, expression and purification of the Der f2 gene

The plasmid pMD19-Der f2, which contained cDNA encoding Der f2, was amplified. The Der f2 coding sequence was inserted into the BamH I–Xho I sites of the pET21 vector. The recombinant Der f2 protein was expressed in the *Escherichia coli* strain BL21 under induction with 1 mM IPTG at 37 °C. The expressed Der f2 protein was purified by Ni–NTA gel affinity chromatography and validated by western blotting.

Construction and expression of the chimeric Fc γ -Der f2 gene

To construct the human Fc γ -Der f2 chimeric gene, we amplified the cDNA encoding Der f2 without the signal peptide by PCR from the pMD19-Der f2 plasmid. The 5'-end primer, which contained a BamH I site, was 5'-GCCGTCT CCGATCCGGTGGAGGCGGTTTCAGGCGGA-3', while the 3'-end primer, which contained a Hind III site, was 5'-GCGTCTCAAGCTTTTAATCACGGATTTTACCATGGGT-3'. After amplification, the PCR products were cloned into the pYD5 vector downstream of the Fc fragment. The sequence was verified to compose 1,185 bp by DNA sequencing. The molecular weight of the recombinant Fc γ -Der f2 fusion protein was determined as 43.4 kD using the Protein MolWt & AA Composition Calculator (ProMACC) software (Protein Information Resource, Washington DC, USA). The expression vector containing the Fc γ -Der f2 chimeric gene was transfected into HEK293 cells. After 72 h, the Fc γ -Der f2 fusion protein was expressed in the cell culture supernatants and purified using an anti-human IgG affinity column.

Western blot analysis of protein expression

The purified proteins were separated on 12.5 % SDS-PAGE gels and transferred to PVDF membranes. For the detection of the Der f2 protein, the membranes were probed with a mouse anti-6 $\times$ His tag antibody followed by an HRP-conjugated goat anti-mouse IgG antibody. The Fc γ -Der f2 fusion protein was detected with an HRP-conjugated goat anti-human IgG antibody. Sera were collected from allergic patients who had experienced anaphylaxis caused

by *Dermatophagoides farinae* that had IgE levels higher than 3 when measured using the ImmunoCap quantitative system (Pharmacia Diagnostics, Uppsala, Sweden). The immunological characteristics of the Der f2 protein and Fc γ -Der f2 fusion protein were analyzed using serum IgE from allergic patients and an HRP-conjugated goat anti-human IgE antibody as described previously [9]. The antigen–antibody complexes on the membranes were visualized using enhanced chemiluminescence reagents (ECL; Amersham Biosciences) and BioMax film (Eastman Kodak).

Generation of a Der f2 airway hypersensitivity mice model

The mice were sensitized with intraperitoneal injections of 20 μ g of Der f2 absorbed to 4 mg of Al(OH)₃ on days 0, 7 and 14. On days 25, 26 and 27, the animals were challenged three times with a daily 30-min dose of Der f2 aerosol, as described previously [10, 11]. Mice in the fusion protein–treated group were injected subcutaneously with Fc γ -Der f2 (20 μ g/animal) on days 2, 12, 25 and 27 according to the protocol of Melendez et al. [12] (see Fig. 1). The Fc γ -Der f2 fusion protein treatment was initiated at the early stage of the sensitization (day 2 after the Der f2 challenge); hence, the sensitization phase has not been completed and followed by other two Der f2 injections on days 7 and 14. Non-sensitized mice challenged with physiological saline (PBS) instead of the fusion protein according to the above treatment schedule were used as normal controls.

Hemi-bronchoalveolar lavage

Mice were killed by exsanguination on day 28. Immediately afterward, the thoracic cavity was opened, and the left hilum of the lung was ligated. Hemi-bronchoalveolar lavage was performed with 3 $\times$ 0.3 ml of Ca²⁺- and Mg²⁺-free PBS supplemented with 0.1 mM EDTA. The bronchoalveolar lavage fluid (BALF) was centrifuged at 1,500 rpm/min for 10 min at 4 °C to separate the BALF from the BAL cells. The BALF samples were stored at

–80 °C until further analysis. The BAL cells were resuspended in 1 ml of PBS, and red blood cells were lysed using an ammonium chloride lysis buffer (Imgenex, San Diego, CA, USA). The total cell number was counted using a hemocytometer. Cyto-centrifuge slide preparations of 50,000 cells were prepared, fixed with methanol, dried and stained with Wright–Giemsa. Differential cell counts were determined for at least 300 cells in random high-power microscopic fields. The percentages of macrophages, neutrophils, eosinophils and lymphocytes out of the total number of cells were calculated. All samples were evaluated in a double-blind manner.

Detection of cytokines and histamine in BALF

Levels of the cytokines IL-5, TNF- α , IL-12p70, IL-4, IL-10, IFN- γ and IL-18 in the BALF were determined using the cytometric bead array method according to the manufacturer's instructions using the FlowCytomix Mouse Basic Kit (Bender Medsystems). The detection limits were 4.0, 2.1, 8.6, 0.7, 5.4, 6.5 and 116.9 pg/ml for IL-5, TNF- α , IL-12p70, IL-4, IL-10, IFN- γ and IL-18, respectively. Concentrations below the detection limits were assumed to be zero for the purpose of statistical analyses. The absolute concentrations of cytokines in the BALF samples were obtained from standard curves. The histamine levels in the BALF samples were measured with an ELISA Kit (Beckman Coulter).

Measurement of murine anti-Der f2 antibodies

The levels of Der f2-specific IgE and IgG in blood samples collected at 12 h after the final Der f2 challenge were determined by ELISA, as described previously [13]. To determine the serum levels of Der f2-specific IgE, 96-well microtiter plates (Coster Corning, NY, USA) were coated with 50 μ g/ml of Der f2 overnight at 4 °C. After washing and blocking with 1 % BSA in PBS, serum samples (1:2 diluted) were added to the wells and incubated for 1 h at 37 °C. Measurement of murine anti-Der f2 antibodies was optimized after performing a series of serum dilutions (data not shown). The plates were then incubated with 1 μ g/ml of an HRP-conjugated goat anti-mouse IgE monoclonal antibody diluted in PBS with 1 % BSA for 0.5 h at 37 °C. After washing, 3,3', 5,5'-tetramethylbenzidine (TMB) substrate (Promega, Madison, WI, USA) with hydrogen peroxide was added, and the absorbances at 450 nm were measured using a microplate reader (Molecular Devices, Menlo Park, CA, USA).

To determine the Der f2-specific IgG levels, plates were coated with 5 μ g/ml of Der f2 and then incubated with serum samples diluted 1:200. For detection, 0.2 μ g/ml of

Protocol:

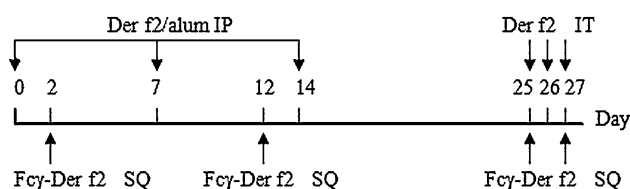


Fig. 1 Treatment schedule and protocol adopted from Melendez et al. [12]

an HRP-conjugated rat anti-mouse IgG monoclonal antibody was used.

Histological analyses

The non-lavaged left lung was removed, inflated with 0.5 ml of 10 % neutral-buffered formalin and fixed in 10 % neutral-buffered formalin for 72 h. The lung was then embedded in paraffin. Sections (5 μ m) were stained with H&E and examined under 20 $\times$ to 100 $\times$ magnification. Peribronchial and perivascular inflammation were assessed by a blinded observer.

Statistical analysis

Statistical analyses were performed using SPSS 11.0 (SAS Institute, Cary, North Carolina, USA). The treatment effects were analyzed by one-way ANOVA followed by a non-parametric test of K independent samples. Values of $P < 0.05$ were considered to indicate significant differences.

Results

Characterization of the purified Der f2 and Fc γ -Der f2 proteins

Upon separation by SDS-PAGE under reducing conditions, the purified Der f2 and Fc γ -Der f2 proteins migrated as single bands of approximately 23 and 43.4 kD, respectively (Fig. 2a, b). SDS-PAGE was closely consistent with the 43.4 kD estimated by ProMACC software. The BSA control appeared as a 66-kD band. Western blot analysis demonstrated that Der f2 and Fc γ -Der f2 were correctly expressed. Der f2 was recognized by an anti-6 $\times$ His tag antibody and Fc γ -Der f2 by an anti-human IgG antibody. Both proteins were detected by *Dermatophagoides farinae*-specific IgE from allergic patients (Fig. 2c, d). These results showed that the Fc γ -Der f2 fusion protein was properly folded to contain a preserved Fc receptor-binding site and was recognized by human IgE antibodies against dust mites.

The inhibition of Der f2-mediated airway allergic reactions by Fc γ -Der f2 fusion protein

Injection of Der f2 allergen in mice stimulated local and systemic allergic reactions, which were not altered by treatment with saline (i.e., treatment control) but the Fc γ -Der f2 fusion protein. In allergic mice, almost 37 % of the total BALF cell number was reduced by the fusion protein (Table 1). Despite the fact that the total cell levels of Fc γ -Der f2-treated allergic mice were four- to fivefold

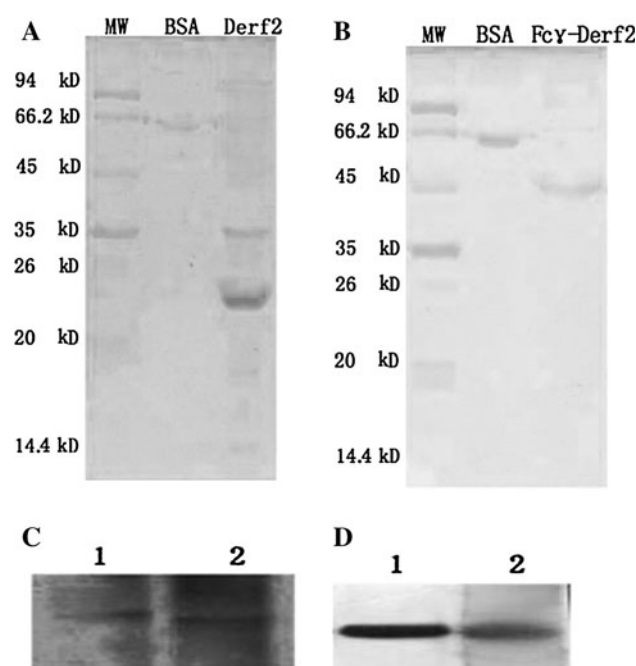


Fig. 2 The purified Der f2 (a) and Fc γ -Der f2 (b) proteins were electrophoresed on a 12.5 % Tris-glycine gel. The molecular weights of the standard marker ladder (lane MW) are indicated in kD on the left. BSA was electrophoresed as a control. c The Der f2 protein was analyzed by western blot with an anti-6 $\times$ His tag antibody (lane 1) and serum IgE from allergic patients specific for *D. farinae* (lane 2). d The Fc γ -Der f2 fusion protein was detected by an anti-human IgG antibody (lane 1) and the serum IgE from allergic patients (lane 2)

Table 1 Der f2-specific serum IgE and IgG levels (absorbances) and total BALF cell numbers ($\times 10^5$)

Group	Serum IgE (OD)	Serum IgG (OD)	BALF total cells ($\times 10^5$)
Control	0.14 $\pm$ 0.01	0.14 $\pm$ 0.18	1.4 $\pm$ 0.40
Sensitized	0.28 $\pm$ 0.05*	2.75 $\pm$ 0.08*	10.9 $\pm$ 1.59*
Fusion protein	0.21 $\pm$ 0.03*/**	2.79 $\pm$ 0.12*	6.5 $\pm$ 0.97*/**

Data are means $\pm$ SD

* $P < 0.01$ versus control mice; ** $P < 0.05$ versus sensitized mice

higher than those of the non-allergic mice, the deviated differential leukocyte count pattern was corrected by the fusion protein to the non-sensitized pattern (Fig. 3a). The Der f2-induced eosinophils and lymphocytes in BALF were significantly ($P < 0.05$) reduced by the fusion protein treatment, hence coordinately up-regulated the relative macrophage percentage. Besides, the fusion protein also remarkably diminished the histamine and cytokines stimulated by the Der f2 allergen (Fig. 3b, c). In comparison with the saline treatment in allergic mice, about 43 % of the BALF histamine was decreased by the fusion protein (Fig. 3b). Cytokine assessment in BALF revealed that a mixed Th1/Th2 pattern was induced after local allergen

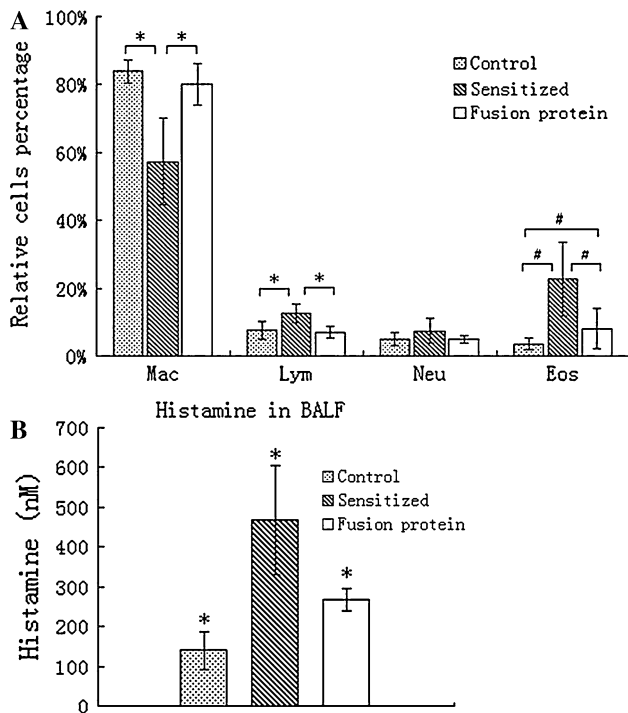


Fig. 3 BALF samples and cells were obtained from Der f2-sensitized and challenged mice in the sensitized group (sensitized, $n = 16$) and fusion protein-treated group (fusion protein, $n = 16$) as well as from non-sensitized mice treated with PBS (control, $n = 8$). The values represent means $\pm$ SD of 8 or 16 mice each group. **a** Differential counts of BAL cells are based on histology. The percentages of macrophages, lymphocytes, neutrophils and eosinophils in the murine BALF samples were determined by counting 300 cells on cytopins stained with Wright-Giemsa using morphological criteria. $*P < 0.01$, $^{\#}P < 0.05$. **b** Concentrations of released histamine in BALF samples from the three groups. $*P < 0.01$, compared to each group. **c** Concentrations of IL-4, IL-5, IL-10, IL-18, IL-12p70, IFN- γ and TNF- α in BALF samples from the three groups. $*P < 0.05$

challenge, showing the elevation of both Th1 cytokines (IFN- γ : 4.3-fold and IL-12p70: 4-fold) and Th2 cytokines (IL-4: 2.3-fold, IL-5: 1.1-fold, IL-10: 2.7-fold and IL-18: 2.8-fold), while these cytokines were significantly ($P < 0.01$) downregulated in the Fc γ -Der f2-treated allergic mice (Fig. 3c). Nonetheless, TNF- α was at lower levels in the sensitized mice than the non-allergic mice, despite the fact that the TNF- α production in the lung was shown to be completely abolished by the fusion protein (Fig. 3c). Serologically, serum Der f2-specific IgE and IgG antibodies were elevated during sensitization, whereas only the IgE isotype but not the IgG was reduced by the fusion protein treatment (Table 1). Histologically, H&E staining of lung tissue sections revealed that mice that had been challenged with Der f2 exhibited moderate pulmonary inflammation, which mainly consisted of eosinophil infiltration around the bronchial and perivascular areas, mucosal goblet cell hyperplasia and the accumulation of

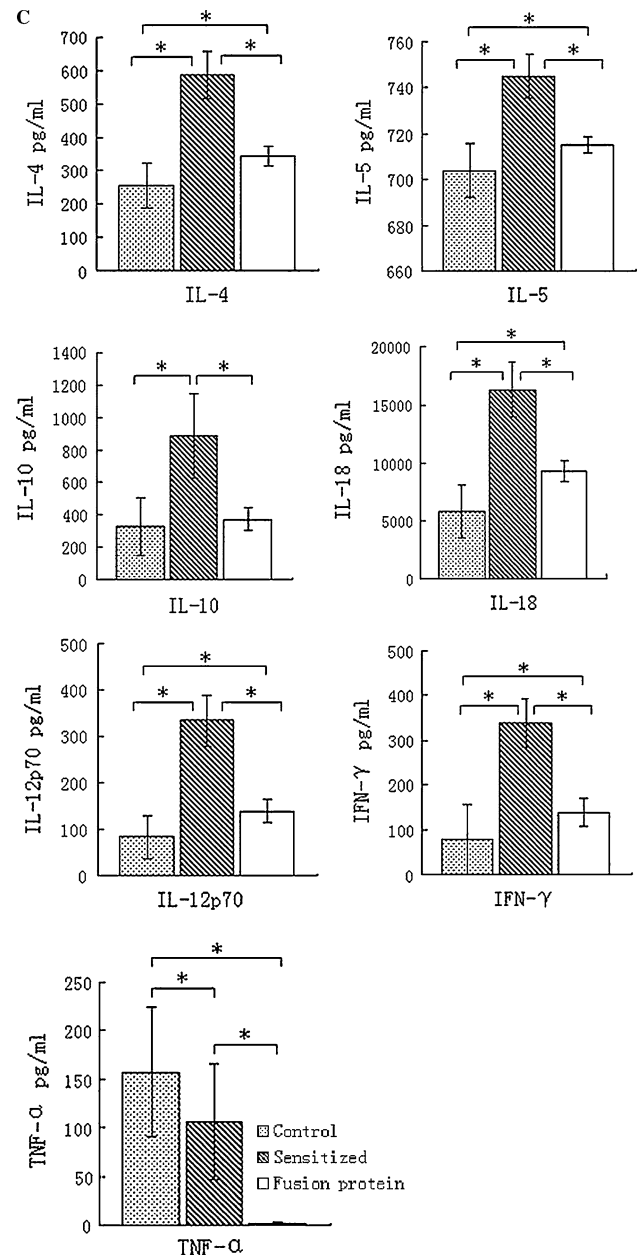


Fig. 3 continued

mucus in portions of the tracheal cavity (Fig. 4). These features were not seen in the non-allergic control mice. Consistent with the BALF findings, treatment of allergic mice with the Fc γ -Der f2 fusion protein was demonstrated to suppress the Der f2-induced accumulation of eosinophils and goblet cell metaplasia in the lungs.

Discussion

The incidence of allergic diseases increases annually, and effective prevention and treatment of anaphylactic

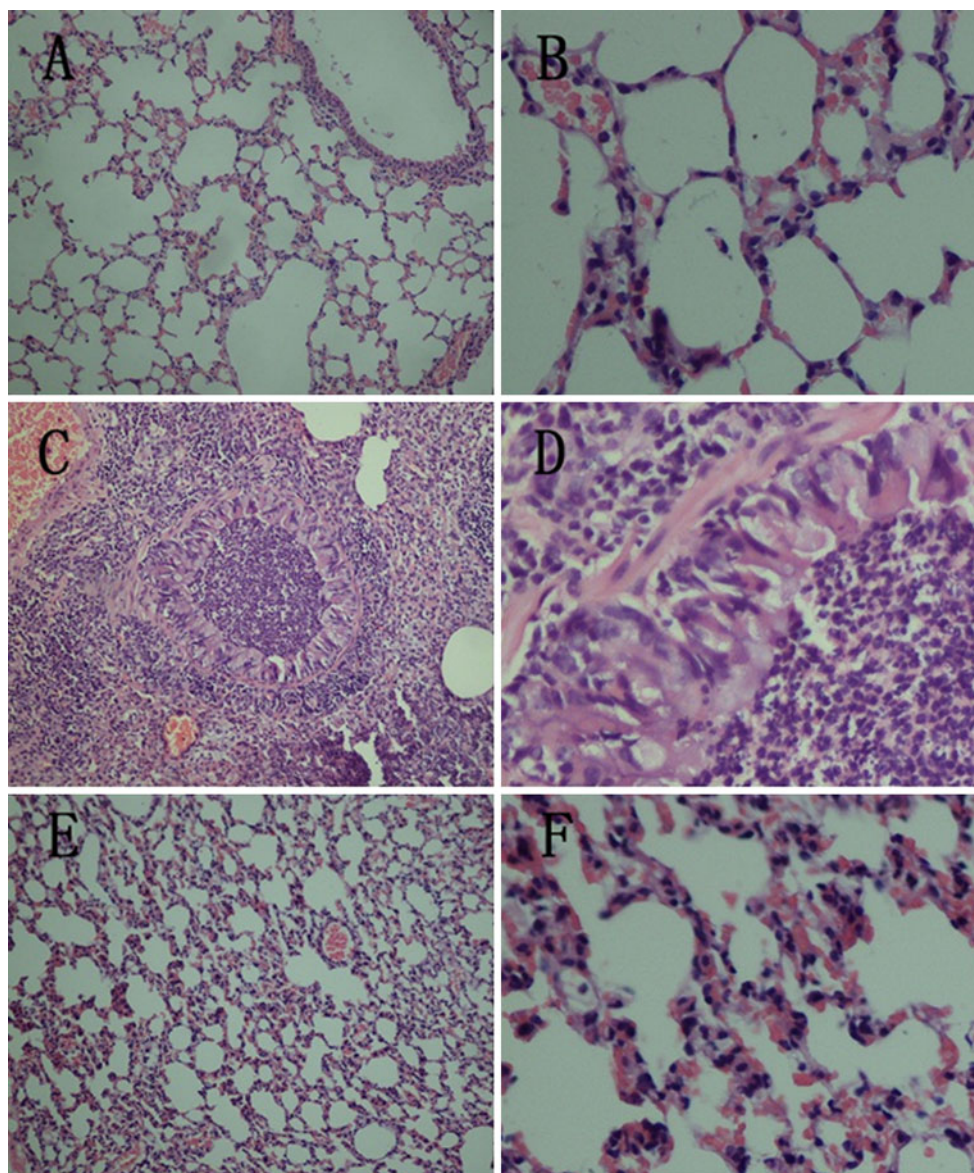


Fig. 4 Light photomicrographs of H&E-stained sections of lung tissues from mice sensitized and challenged with Der f2 with (e, f) or without (c, d) the Fc γ -Der f2 fusion protein and from non-sensitized

mice treated with PBS (a, b). The histological changes to the lung tissue were examined at $\times 20$ (a, c, e) or $\times 100$ (b, d, f) magnification

responses are highly desirable. Currently, a number of approaches for immunotherapy are being developed, including the use of monoclonal antibodies [9] and allergen-peptide desensitization to modulate the immune response. Efforts have been made toward the prevention and/or treatment of allergic reactions based on pathogenesis. In mast cell, the tyrosine phosphorylation of Syk, ERK and several other cellular substrates was reduced in response to the activation of the inhibitory Fc γ RIIb. However, tyrosine phosphorylation of the adapter protein downstream of kinase (Dok), growth factor receptor-bound protein 2 (Grb2) and SH2 domain-containing inositol 5-phosphatase (SHIP), all of which silence the Fc ϵ RI-mediated activation pathway, was increased [14].

The successes with GE2 and GFD took advantage of the inhibitory capacity of Fc γ RIIb in blocking allergic responses in vitro and in animal models and provided hope for the use of these molecules as potential novel treatments for human allergic diseases. In seeking optimal candidates for further drug development, we constructed the Fc γ -Der f2 fusion protein, which was able to inhibit allergic reactions to *Dermatophagoides farinae* in a mouse model. This construction was based on evidence that the murine Fc γ Rs can bind to human IgG Fc [15]. Therefore, the human Fc portion of the fusion protein could be expected to bind to the murine Fc γ Rs, including to Fc γ RIIb, which contains an ITIM inhibitory signaling motif. On the other hand, the Der

f2 portion will bind to murine Der f2-specific IgE and/or IgG on the surface of basophils and mast cells in the sensitized mice. Fc γ RI and Fc γ RIII were Fc γ R that containing ITAM, and thus pose the potential to induce degranulation in manner similar to that observed following Fc ϵ RI aggregation [16, 17]. Considering that both Fc γ RI and Fc γ RIII are not constitutively expressed in human and rodent mast cells [17], but expressed Fc γ RII [18, 19], it is speculated that the inhibitory effects of Fc γ -Der f2 fusion protein on mast cells and basophils were exerted by the co-aggregation of Fc ϵ RI and the inhibitory Fc γ IIb. And hence, Fc γ RIII pathway is not suggested to be activated by the Fc γ -Der f2 fusion protein. However, in the present study, only indirect evidence of BALF histamine measurement was provided to support the activation and degranulation of mast cells and basophils. Further experiments on isolated mast cells from lung tissues are required to study the degranulation state before this hypothesis can be established. Additionally, an alternative explanation could be the formation of soluble immune complexes that leading to decreased soluble antigen and an attenuated CD4⁺ Th2 response [20]. And this will be addressed by using Fc γ R^{-/-} mice model in separate study. Given that allergen-specific immune response could also be inhibited by the subcutaneous application of the allergen itself, as demonstrated in mouse model of hypoallergenic variants of parvalbumin (SIT) [21]. Although it is unlikely to happen in the current acute model of airway allergy, in order to exclude this, a group of mice being immunized subcutaneously with an equimolar amount of Der f2 protein will be introduced in the future study.

Analyses of Der f2-specific IgG and IgE levels in the sensitized mice revealed that the sensitization and challenge induced significant increases in the serum levels of Der f2-specific antibodies. However, we found that the IgE levels in mice during or just after the anaphylactic reaction were relatively low compared with IgG levels. An important factor is probably the fact that an anaphylactic reaction is caused by IgE binding to mast cells; however, the ELISA only measures the levels of free IgE molecules in the serum. Mast cells may be sufficiently sensitized to trigger a reaction after allergen binding, even if the free IgE is very low in serum [22]. A possible related phenomenon has been reported in humans, since it has been observed that some individuals become temporarily negative in IgE tests after a systemic anaphylactic reaction, probably because IgE is located extravascularly, bound in immune complexes or on mast cells [23, 24]. However, the half-life of the Der f2-specific IgE and IgG should also be taken into account. Moreover, IgG1 antibodies can also be anaphylactic in mice. The simultaneous operation of IgG- and IgE-mediated pathways leads to severe anaphylaxis [25]. In the future study, IgG subtypes should be determined to

reveal whether there is any shift between the Th2-associated IgG1 production and Th1-associated IgG2 following the Der f2 challenge and Fc γ -Der f2 fusion protein treatment.

In terms of cytokine repertoire, Th1 cells produce IFN- γ and IL-12, while Th2 cells produce IL-4, IL-5, IL-6 and IL-10. However, the most remarkable increase in cytokine observed after Der f2 challenge was IL-18, which can be produced by immune and non-immune cells that regulate both Th1 and Th2 responses [26]. It was documented that IL-18 alone can trigger Th2 immune responses, while in collaboration with IL-12, it enhances Th1-mediated immune responses to induce IFN- γ [27, 28]. Thus, this explains why a mixed pattern of Th1 and Th2 response was observed in the present study. No doubt, Th2 immune responses play a significant role in airway allergies that flavor the humoral response to synthesis immunoglobulin like IgE and IgG [29]. However, inflammatory and anaphylactic reactions in allergies require the driving force of Th2 cell-mediated immune response [28, 30]. The Fc γ -Der f2 fusion protein was shown to be effective in attenuating the Th1 and Th2 cytokines secretion, and suggesting its immunomodulatory roles in Der f2-specific allergies. Nonetheless, it is controversial that a complete abolishment of TNF- α was observed in the allergic mice treated with the fusion protein, in addition to the observation that TNF- α level measured in the allergic mice was lower than that of the non-allergic mice. It was reported that the severities of antigen-induced airway hypersensitivity and eosinophilic airway inflammation were correlated with the expression of TNF- α and its receptor [31]. But in another study, mean concentration of TNF- α in nasal secretions were shown to have no significant difference between patients with perennial nasal allergy and healthy subjects, although mRNA of TNF- α expression could be stimulated after allergen challenge in vitro [32]. This demands further elucidation.

In this study, we evaluated the ability of the Fc γ -Der f2 fusion protein to block allergic responses using a variety of experimental systems. The levels of released inflammatory mediators and the percentage of eosinophils in BALF samples were significantly inhibited by the Fc γ -Der f2 fusion protein. The fusion protein also exhibited strong inhibitory effects on the pathological changes observed in mice sensitized with Der f2. One important improvement after Der f2 challenge in the fusion protein-treated allergic mice was decreased mucus production by epithelial cells, which was associated with mild goblet cell hyperplasia/metaplasia.

Our results also showed that short-term treatment with the Fc γ -Der f2 fusion protein as an immunotherapy did not induce any effector allergic responses, presumably because of its ability to co-aggregate Fc γ RIIb with Fc ϵ RI on

basophils and mast cells. Long-term immunotherapy with this Fc γ -Der f2 fusion protein in the mouse model was not investigated because we believe the fusion protein could eventually induce anti-human Fc γ responses that may complicate the results and their interpretation. In summary, the Fc γ -Der f2 fusion protein was demonstrated to function as an effective immunotherapeutic agent. Treatment with Fc γ -allergen proteins such as GFD and Fc γ -Der f2 is predicted to have enhanced safety because they are unable to trigger responses in mast cells and basophils. Therefore, chimeric human Fc γ -allergen proteins provide a new platform for antigen-specific immunotherapy for the treatment of a host of human allergic diseases.

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