



# The development of a candidate of desensitization vaccines against Der f 2 nearly without IgE-binding activity

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## ABSTRACT

Considering the important role of Der f 2 in house dust mites mediating allergic diseases and allergic adverse effects during allergen-specific immunotherapy (AIT), we intend to develop a candidate of desensitization vaccines against Der f 2 without allergenicity. According to the reported immunoglobulin E (IgE)-binding B and T cell epitopes of Der f 2, four candidates of desensitization vaccines against Der f 2 were developed. Recombinant wild-type Der f 2 (rWt-Der f 2) preserved conformational and linear IgE-binding B epitopes. rWt-Der f 2 linearized by reduction and alkylation reactions (rWt-Der f 2 (red/alk)) and recombinant modified-type Der f 2 (rMt-Der f 2) were developed via destroying conformational and linear IgE-binding B epitopes respectively. rMt-Der f 2 linearized by reduction and alkylation reactions (rMt-Der f 2 (red/alk)) was developed by destroying conformational and linear IgE-binding B epitopes. T cell epitopes of 4 candidates were preserved. The change of their IgE-binding activity was determined by enzyme linked immunosorbent assay (ELISA), western blot and inhibition ELISA. Compared with rWt-Der f 2, the IgE-binding activity of rWt-Der f 2 (red/alk), rMt-Der f 2 and rMt-Der f 2 (red/alk) all decreased, which was consistent with the result of western blot. The IgE-binding activity of rMt-Der f 2 and rMt-Der f 2 (red/alk) was not significantly different ( $P = 0.0863 > 0.05$ ), which was comparable to that of their corresponding negative controls ( $P = 0.3488$  and  $0.4459$ , both  $> 0.05$ ). The result of inhibition ELISA also showed that their IgE-binding activity decreased, and rMt-Der f 2 (red/alk) was the lowest. Conclusively, we developed the candidate of desensitization vaccines against Der f 2, rMt-Der f 2 or rMt-Der f 2 (red/alk), nearly without allergenicity, which would potentially prevent HDM allergic patients from allergic adverse effects caused by AIT.

**Abbreviations:** HDM(s), house dust mite(s); IgE, immunoglobulin E; IgEs, immunoglobulin E antibodies; AIT, allergen-specific immunotherapy; FcεRI, high affinity IgE receptors; Cys, cysteine; rWt-Der f 2, recombinant wild-type Der f 2; DTT, dithiothreitol; IAA, iodoacetamide; rWt-Der f 2 (red/alk), recombinant wild-type Der f 2 linearized by reduction and alkylation reactions; rMt-Der f 2, recombinant modified-type Der f 2; rMt-Der f 2 (red/alk), recombinant modified-type Der f 2 linearized by reduction and alkylation reactions; ELISA, enzyme linked immunosorbent assay.

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## 1. Introduction

As one of the most common indoor allergens throughout the world, house dust mites (HDMs) are reported to be associated with various allergic diseases, such as allergic asthma, rhinitis and atopic dermatitis (Yuuki et al., 1991, Chua et al., 1996, Ichikawa et al., 2005, Yang et al., 2012), which are a causative factor of more than 50% allergic patients (Chen et al., 2012). Der f 2, which belongs to the group 2 of major HDM allergens (Haida et al., 1985, Platts-Mills and Chapman, 1987, Yang et al., 2012), affects over 90% HDM allergic patients (Baldo and Donovan, 1990). Moreover, immunoglobulin E antibodies (IgEs) against Der f 2 account for more than one-third of those against whole HDM crude extracts in sera of HDM allergic patients (Heymann et al., 1989, Lynch et al., 1997). So, Der f 2 plays a critical role in HDMs mediating allergic diseases.

Up to now, allergen-specific immunotherapy (AIT), which is performed by repeated injection and progressively increased dose of disease-causing allergens over a long time to induce the immune tolerance, is the only curative method for allergic diseases (Takai et al., 1999, Yang et al., 2012, Tulaeva et al., 2020). However, as the biggest disadvantage of AIT (Takai et al., 1999, Johannessen et al., 2005), allergic adverse effects can widely limit the use of AIT (Faniel et al., 2018). To decrease the incidence of allergic adverse effects caused by AIT, researchers are devoted to developing desensitization vaccines with reduced allergenicity (Takai et al., 1999, Johannessen et al., 2005).

When multivalent allergens crosslinking to high affinity IgE receptors (FcεRI) occupied by IgEs on mast cells or basophils, allergic responses are triggered, resulting in cellular degranulation and subsequent mediator release such as histamine (Metzger et al., 1986, Takai et al., 2001). The binding sites of allergens and IgEs, named as IgE-binding B epitopes, are mainly responsible for allergic adverse effects caused by AIT (Nishiyama et al., 1999, Haka et al., 2019, Tscheppe et al., 2020). On the other hand, T cell epitopes of allergens are critical for inducing the immune tolerance via generating regulatory T cells and increasingly secreting inhibitory cytokines, such as interleukin-10, cytotoxic T lymphocyte antigen-4, programmed death-1 and transforming growth factor-β (Jutel et al., 2006, Akdis and Akdis, 2007). Therefore, desensitization vaccines with reduced allergenicity can be developed by destroying IgE-binding B epitopes responsible for allergic adverse effects and preserving T cell epitopes (Nishiyama et al., 1999, Haka et al., 2019, Tscheppe et al., 2020).

To date, IgE-binding B and T cell epitopes of Der f 2 have been well studied (Cui et al., 2016; Ichikawa et al., 1998, 2005; Liu and Lin, 1998; Nishiyama et al., 1999; Takai et al., 1997b, 2001). The reported IgE-binding B epitopes of Der f 2 included the following amino acid fragments: 69–78, 73, 75, 78, 79, 127–129 (Ichikawa et al., 1998, Liu and Lin, 1998, Nishiyama et al., 1999, Takai et al., 2001, Ichikawa et al., 2005); 15–22, 80–89, 106–113 (Cui et al., 2016); 1–24, 25–29, 67–90, 121–123 (Takai et al., 1997b). Amino acid fragments, 1–8 and 119–129, possibly contained T cell epitopes (Ichikawa et al., 1998). It was also reported that conformational rather than linear IgE-binding B epitopes were mainly responsible for IgE-binding activity of Der f 2 (Chua et al., 1991, van 't Hof et al., 1991, Smith and Chapman, 1996, Derewenda et al., 2002). Moreover, conformational IgE-binding B epitopes of Der f 2 primarily depended on intramolecular disulfide bonds, particularly in Cysteine (Cys)8–Cys119, Cys73–Cys78 (Lombardero et al., 1990, Nishiyama et al., 1993, Nishiyama et al., 1995, Takai et al., 1997a; Takai et al., 1997b). Thus, destroying intramolecular disulfide bonds to achieve widely destructive conformational IgE-binding B epitopes of Der f 2 is the common method for developing desensitization vaccines against Der f 2 with reduced allergenicity (Takai et al., 1997a; Takai et al., 1997b, Yasue et al., 1998, Takai et al., 2000a; Takai et al., 2000b, Yang et al., 2012). However, the possibility that the remaining IgE-binding B epitopes of Der f 2 still give rise to allergic adverse effects during AIT can't be excluded. It was reported that the hypoallergenic Der p 2 vaccine developed by destroying the disulfide bond (Cys8–Cys119), was still

able to bind to IgEs (Asturias et al., 2009). Recently, the desensitization vaccine against Ara h 2 without allergenicity has been successfully developed via removing conformational and linear IgE-binding B epitopes and persevering T cell epitopes (Tscheppe et al., 2020), which has not been applied for Der f 2.

In this study, we developed the candidate of desensitization vaccines against Der f 2, nearly without allergenicity, via destroying conformational and linear IgE-binding B epitopes and preserving T cell epitopes, which would potentially prevent HDM allergic patients from adverse effects caused by AIT.

## 2. Methods and materials

### 2.1. Serum samples

The IgE concentration of patients' sera for this study was detected by Phadia 1000 Immunoassay Analyzers (Phadia, Uppsala, Sweden). The inclusion criteria: IgEs specific to HDMs (code: D2) were over 0.35 kU/L. Finally, forty-two sera of HDM allergic patients (Table S1) and 5 sera of non-allergic individuals as negative controls were included in the study. The study was conducted under the guidance of the Helsinki Declaration and approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University.

### 2.2. Preparation of 4 candidates of desensitization vaccines against Der f 2

According to the reported studies about Der f 2, the amino acid fragments (69–78, 73, 75, 78, 79, 127–129 (Ichikawa et al., 1998, Liu and Lin, 1998, Nishiyama et al., 1999, Takai et al., 2001, Ichikawa et al., 2005); 15–22, 80–89, 106–113 (Cui et al., 2016); 1–24, 25–29, 67–90, 121–123 (Takai et al., 1997b) formed IgE-binding B epitopes. Amino acid sequences, 1–8, 119–129, possibly contained T cell epitopes (Ichikawa et al., 1998). As reported, α-helices were critical for the secondary structures of Der f 2, therefore, which were also preserved (Johannessen et al., 2005). These amino acid fragments forming β chain of Der f 2 included VKD, IKKVM, CIIH, PFTLEALFD, KIEIKASL, LEI, QYDIKYTN, VVVTVKLIG, GVLACAIA, GKI, which was not possibly related to its allergenicity and immunogenicity (Ichikawa et al., 2005). Subsequently, the recombinant modified-type Der f 2 (rMt-Der f 2) plasmid was constructed via preserving nucleotide sequences encoding T cell epitopes and α-helices of Der f 2, and removing nucleotide sequences encoding IgE-binding B epitopes.

The recombinant wild-type Der f 2 (rWt-Der f 2) gene was cloned to between Xho I and Nco I of pET-28a plasmids by the one-step-clone kit (Vazyme, Nanjing, China) to construct rWt-Der f 2-recombinant plasmid. The designed rMt-Der f 2 plasmid was synthesized by the Genscript Biotechnology Corporation (Nanjing, China). Then, the rWt-Der f 2-recombinant plasmid and rMt-Der f 2-recombinant plasmid were transferred into *Escherichia coli* (*E. coli*) (BL21 DE3) (Vazyme, Nanjing, China). rWt-Der f 2 and rMt-Der f 2 proteins were expressed with the addition of isopropyl-β-D-thiogalactoside at final concentration of 1 mM, and were purified by high-affinity Ni<sup>2+</sup> NTA resin (Genscript, Nanjing, China). Then, purified rWt-Der f 2 and rMt-Der f 2 proteins were further linearized by reduction and alkylation reactions according to Tscheppe's study (Tscheppe et al., 2020), which could cause the destruction of conformational IgE-binding B epitopes of rWt-Der f 2 and rMt-Der f 2. Briefly, rWt-Der f 2, rMt-Der f 2 proteins with the addition of dithiothreitol (DTT) (Sangon, Shanghai, China) at final concentration of 50 mM were incubated at 60 °C for 1 h. Subsequently, protein samples with the addition of iodoacetamide (IAA) (Sangon, Shanghai, China) at final concentration of 200 mM were incubated at room temperature for 2 h. Then, DTT at final concentration of 37 mM was added to inactivate unbound IAA, which was incubated for 20 min. Then, protein samples were dialyzed against PBS. Finally, four candidates were developed, including rWt-Der f 2, rWt-Der f 2 linearized by reduction and alkylation

reactions (rWt-Der f 2 (red/alk)), rMt-Der f 2 and rMt-Der f 2 linearized by reduction and alkylation reactions (rMt-Der f 2 (red/alk)).

### 2.3. The reactivity of 4 candidates of desensitization vaccines against Der f 2 to IgEs from sera of HDM allergic patients

The IgE-binding activity of 4 candidates of desensitization vaccines against Der f 2 was firstly determined by enzyme linked immunosorbent assay (ELISA). rWt-Der f 2, rWt-Der f 2 (red/alk), rMt-Der f 2 and rMt-Der f 2 (red/alk) proteins (all 1 µg/well) were coated to the microtiter plate (Corning Costar, Massachusetts, USA) at 4 °C overnight. Considering that the group 2 mite allergens were present at high concentration in mite feces (Yang et al., 2012), HDM crude extracts (1 µg/well) were used as the positive control. Five sera of non-allergic individuals were used as negative controls. The first antibody was 42 sera of HDM allergic patients and 5 sera of non-allergic individuals (1:10 diluted in PBS-0.1% Tween 20). Anti-IgEs linked with horseradish peroxidase (HRP) (KPL, Maryland, USA) as the second antibody (1:2500 diluted in PBS-0.1% Tween 20) were used to detect IgEs binding to rWt-Der f 2, rWt-Der f 2 (red/alk), rMt-Der f 2 and rMt-Der f 2 (red/alk) proteins. The reaction was visualized by 100 µL of 3', 5', 5'-tetramethylbenzidine substrate (TMB) (Beyotime, Shanghai, China) for 10 min in dark, and was stopped by 50 µL of 2 M H<sub>2</sub>SO<sub>4</sub>. Their absorbance values were read at 450 nm by the Multiskan GO (Thermo Fisher Scientific, Massachusetts, USA). The cut-off values were presented as the average (negative controls) + 3 × standard deviation (negative controls).

### 2.4. Western blot and inhibition ELISA

The IgE-binding activity of 4 candidates was further determined by western blot and inhibition ELISA. For western blot, 10 µg respective proteins (rWt-Der f 2, rWt-Der f 2 (red/alk), rMt-Der f 2 and rMt-Der f 2 (red/alk)) after electrophoresis were blotted to the 0.22 µm polyvinylidene difluoride membrane (Merck Millipore, Massachusetts, USA). The first antibody was pooled sera of HDM allergic patients (1:10 diluted in PBS-0.1% Tween 20), and the second antibody was anti-IgEs linked with HRP (1:5000 diluted in PBS-0.1% Tween 20) (KPL, Maryland, USA). The IgEs binding to rWt-Der f 2, rWt-Der f 2 (red/alk), rMt-Der f 2 and rMt-Der f 2 (red/alk) proteins were visualized by Immobilon<sup>TM</sup> Western HRP substrate luminol reagent (Merck Millipore, Massachusetts, USA) using Tanon 5200 multi-imaging system (Tanon, Shanghai, China).

For inhibition ELISA, HDM crude extracts (1 µg/well) were coated to the microtiter plate (Corning Costar, Massachusetts, USA) at 4 °C overnight. Before added to the microtiter plate, sera of HDM allergic patients (1:20 diluted in PBS-0.1% Tween 20) were preincubated with HDM crude extracts, rWt-Der f 2, rWt-Der f 2 (red/alk), rMt-Der f 2 and rMt-Der f 2 (red/alk) at 37 °C for 1 h. The inhibitory concentration of HDM crude extracts, rWt-Der f 2, rWt-Der f 2 (red/alk), rMt-Der f 2 and rMt-Der f 2 (red/alk) was 10 µg/mL or 20 µg/mL. The subsequent steps were same to those described in Section 2.3. The group of HDM crude extracts inhibited by HDM crude extracts was the positive control, and sera of non-allergic individuals was the negative control.

### 2.5. Statistical analysis

The OD<sub>450nm</sub> values of HDM crude extracts, rWt-Der f 2, rWt-Der f 2 (red/alk), rMt-Der f 2 and rMt-Der f 2 (red/alk) reactions to IgEs from sera of HDM allergic patients were compared by paired *t* test (*n* = 42) (GraphPad Prism 7). The OD<sub>450nm</sub> values of rMt-Der f 2, rMt-Der f 2 (red/alk) (*n* = 42) and their corresponding negative controls (*n* = 5) were compared by unpaired *t* test (GraphPad Prism 7). *P* < 0.05 was considered as statistically significant, \*, *P* < 0.05; \*\*, *P* < 0.01; \*\*\*, *P* < 0.001.

## 3. Results

### 3.1. Design of the candidate of desensitization vaccines against Der f 2

According to the reported studies about Der f 2, the amino acid sequences forming IgE-binding B, T cell epitopes and key structures were shown in Table 1. How to develop the rMt-Der f 2 was showed in Fig. 1.

### 3.2. Preparation of 4 candidates of desensitization vaccines against Der f 2

The rWt-Der f 2 and rMt-Der f 2 proteins expressed by *E. coli* were resolved on 15% SDS-PAGE gel, which sited at approximately 14 kDa and 11 kDa respectively (Fig. 2). Moreover, rWt-Der f 2 (red/alk) and rMt-Der f 2 (red/alk) were slightly higher than rWt-Der f 2 and rMt-Der f 2 respectively (Fig. 2), which could be explain that linearized proteins encountered with a bigger resistance during electrophoresis. Moreover, the molecular weights of rWt-Der f 2 (red/alk) or rMt-Der f 2 (red/alk) were same at non-reducing and reducing condition (Fig. 2), which indirectly indicated that rWt-Der f 2 (red/alk) and rMt-Der f 2 (red/alk) were linearized.

### 3.3. The reactivity of 4 candidates of desensitization vaccines against Der f 2 to IgEs from sera of HDM allergic patients

The reactivity of 4 candidates of desensitization vaccines against Der f 2 to IgEs from sera of HDM allergic patients was firstly determined by ELISA (Fig. 3). According to the result of ELISA, the reactivity of HDM crude extracts and rWt-Der f 2 to IgEs from sera of HDM allergic patients was not statistically different (Median = 0.71 and 0.77 respectively; *P* > 0.05; Fig. 3a-1). Compared with rWt-Der f 2, the reactivity of rWt-Der f 2 (red/alk), rMt-Der f 2 and rMt-Der f 2 (red/alk) to IgEs from sera of HDM

**Table 1**

The amino acid sequences forming IgE-binding B, T cell epitopes and key structures of Der f 2.

| Epitopes/key structures                                                  | Amino acid sequences                                                            | References                                                                                                    |
|--------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|
| IgE-binding B epitopes                                                   |                                                                                 |                                                                                                               |
| 69-78, 73, 75, 78, 79, 127-129                                           | DTNACHFVKC, C, F, C, P, IRD                                                     | (Ichikawa et al., 1998, Liu and Lin, 1998, Nishiyama et al., 1999, Takai et al., 2001, Ichikawa et al., 2005) |
| 15-22, 80-89, 106-113                                                    | KVMVDGCH, LVKQGYDIK, VTVKLIGD                                                   | (Cui et al., 2016)                                                                                            |
| 1-24, 25-29, 67-90, 121-123                                              | DQVDVKDCANNEIKVMVDGCHGS, DPCII, GIDTNACHFVKCPLVKQGYDIKY, IAT                    | (Takai et al., 1997b)                                                                                         |
| T epitopes                                                               |                                                                                 |                                                                                                               |
| 1-8, 119-129                                                             | DQVDVKDC, CAIATHGKIRD                                                           | (Ichikawa et al., 1998)                                                                                       |
| α-helices                                                                |                                                                                 |                                                                                                               |
| 71-75                                                                    | NACHF                                                                           | (Johannessen et al., 2005)                                                                                    |
| β chain                                                                  |                                                                                 |                                                                                                               |
| 5-7, 13-17, 27-30, 34-42, 51-58, 61-63, 85-93, 104-112, 115-122, 125-127 | VKD, IKKVM, CIIH, PFTLEALFD, KIEIKASL, LEI, QYDIKYTNW, VVVTVKLIG, GVLACAIA, GKI | (Ichikawa et al., 2005)                                                                                       |
| Disulfide bonds                                                          |                                                                                 |                                                                                                               |
| 8/119, 21/27, 73/78                                                      | Cys8Cys119, Cys21Cys27, Cys73Cys78                                              | (Lombardero et al., 1990; Nishiyama et al., 1993, 1995, Takai et al., 1997a, b)                               |

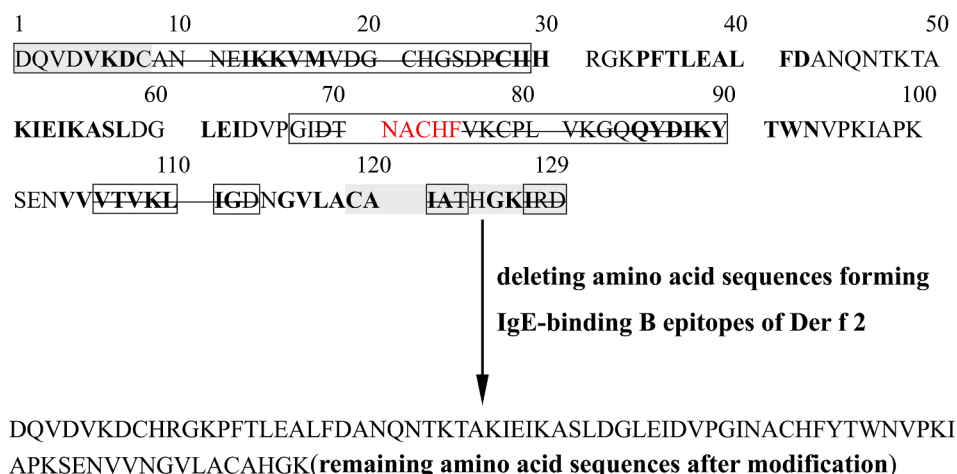


**Fig. 1.** Design of the candidate of desensitization vaccines against Der f 2. The reported and deleted amino acid sequences forming IgE-binding B epitopes of Der f 2 were marked by boxes and strikethroughs respectively. Amino acid sequences marked in grey formed T cell epitopes, and amino acid sequences marked in red formed the  $\alpha$ -helices of Der f 2. The bold amino acid sequences formed the  $\beta$  chain of Der f 2.

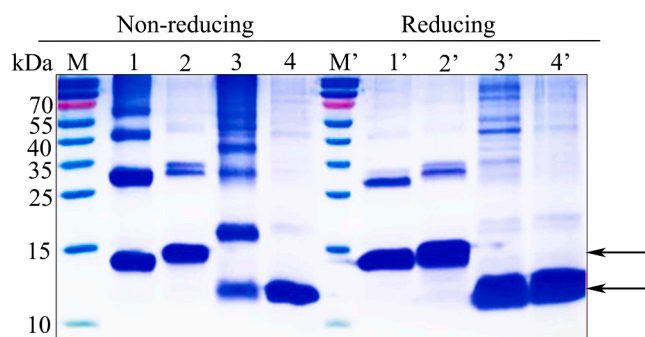


**Fig. 2.** Preparation of 4 candidates of desensitization vaccines against Der f 2. Lane M, M' - Protein marker. 1, 2, 3, 4 - represented rWt-Der f 2, rWt-Der f 2 (red/alk), rMt-Der f 2, rMt-Der f 2 (red/alk) respectively at the non-reducing condition; 1', 2', 3', 4' - represented rWt-Der f 2, rWt-Der f 2 (red/alk), rMt-Der f 2, rMt-Der f 2 (red/alk) respectively at the reducing condition. The above arrow represented rWt-Der f 2/rWt-Der f 2 (red/alk), and the below arrow represented rMt-Der f 2/rMt-Der f 2 (red/alk).

allergic patients significantly decreased (Median = 0.19, 0.12 and 0.13 respectively; all  $P < 0.001$ ; Figs. 3a-2, 3a-3, 3a-4). Further, the reactivity of rWt-Der f 2 (red/alk) and rMt-Der f 2 to IgEs from sera of HDM allergic patients was significantly different ( $P < 0.001$ ; Fig. 3a-5), and rMt-Der f 2 had lower IgE-binding activity. Nevertheless, the reactivity of rMt-Der f 2 and rMt-Der f 2 (red/alk) to IgEs from sera of HDM allergic patients was not significantly different (Median = 0.12 and 0.13 respectively;  $P > 0.05$ ; Fig. 3a-6). Among 42 HDM allergic patients, rMt-Der f 2 and rMt-Der f 2 (red/alk) were respectively recognized by IgEs from 13/42 (31%) and 5/42 (12%) HDM allergic patients' sera. Moreover, IgE-binding activity of rMt-Der f 2 and rMt-Der f 2 (red/alk) in 42 HDM allergic patients was comparable to that in 5 non-allergic individuals (both  $P > 0.05$ ; Fig. 3b-1, Fig. 3b-2), which possibly meant that rMt-Der f 2 and rMt-Der f 2 (red/alk) nearly had no IgE-binding activity.

### 3.4. Western blot and inhibition ELISA

According to the result of western blot, compared with rWt-Der f 2, rWt-Der f 2 (red/alk) and rMt-Der f 2, rMt-Der f 2 (red/alk) showed the lowest IgE-binding activity (Fig. 4a), which was consistent with the result of ELISA.

According to the result of inhibition ELISA, the inhibitory rate of HDM crude extracts to HDM crude extracts was approximately 80% and

90% at inhibitory concentration of 10  $\mu$ g/mL (Fig. 4b-1) and 20  $\mu$ g/mL (Fig. 4b-2) respectively. Among most patients, the inhibitory rate of rWt-Der f 2, rWt-Der f 2 (red/alk), rMt-Der f 2 and rMt-Der f 2 (red/alk) to HDM crude extracts orderly decreased at inhibitory concentration of 10  $\mu$ g/mL (Fig. 4b-1) or 20  $\mu$ g/mL (Fig. 4b-2). The result indirectly showed that the reactivity of rWt-Der f 2, rWt-Der f 2 (red/alk), rMt-Der f 2 and rMt-Der f 2 (red/alk) to IgEs from sera of HDM allergic patients also orderly decreased, and rMt-Der f 2 was the lowest.

### 4. Discussion

Der f 2, which belongs to the group 2 of major HDM allergens, affects over 90% HDM allergic patients (Haida et al., 1985, Platts-Mills and Chapman, 1987, Yang et al., 2012). Additionally, the use of AIT is widely limited by allergic adverse effects (Takai et al., 1999, Johansson et al., 2005). Therefore, in the study, we developed the candidate of desensitization vaccines against Der f 2 without IgE-binding activity via destroying conformational and linear IgE-binding B epitopes and preserving T cell epitopes, which would potentially prevent HDM allergic patients from allergic adverse effects during AIT.

According to the result of ELISA, the reactivity of rMt-Der f 2 and rMt-Der f 2 (red/alk) to IgEs from sera of HDM allergic patients was comparable to that of non-allergic individuals ( $P > 0.05$ ). According to the result of inhibition ELISA, rMt-Der f 2 (red/alk) had no inhibitory effect on HDM crude extracts at inhibitory concentration of 10  $\mu$ g/mL and 20  $\mu$ g/mL. Collectively, the IgE-binding activity of rMt-Der f 2 and rMt-Der f 2 (red/alk) was remarkably reduced or even absent. The common methods for developing desensitization vaccines against Der f 2 with reduced allergenicity was to destroy intramolecular disulfide bonds, further causing widely destructive conformational IgE-binding B epitopes of Der f 2 (Takai et al., 1997a; Takai et al., 1997b, Yasue et al., 1998, Takai et al., 2000a; Takai et al., 2000b, Yang et al., 2012). It was reported that the hypoallergenic Der p 2 vaccine, which was developed by destroying the disulfide bond (Cys8-Cys119), was still able to bind to IgEs (Asturias et al., 2009). Combined with the above, the hypoallergenic Der f 2 vaccines developed by only destroying intramolecular disulfide bonds of Der f 2 possibly remained IgE-binding activity.

Linear IgE-binding B epitopes also played a critical role in Der f 2 mediating IgE-binding responses. In the study, we developed 4 candidates of desensitization vaccines against Der f 2. The rWt-Der f 2 preserved conformational and linear IgE-binding B epitopes. The rWt-Der f 2 (red/alk) was linearized by reduction and alkylation reactions, which meant that its conformational IgE-binding B epitopes were destroyed. Therefore, the decrease of IgE-binding activity of rWt-Der f 2 to rWt-Der

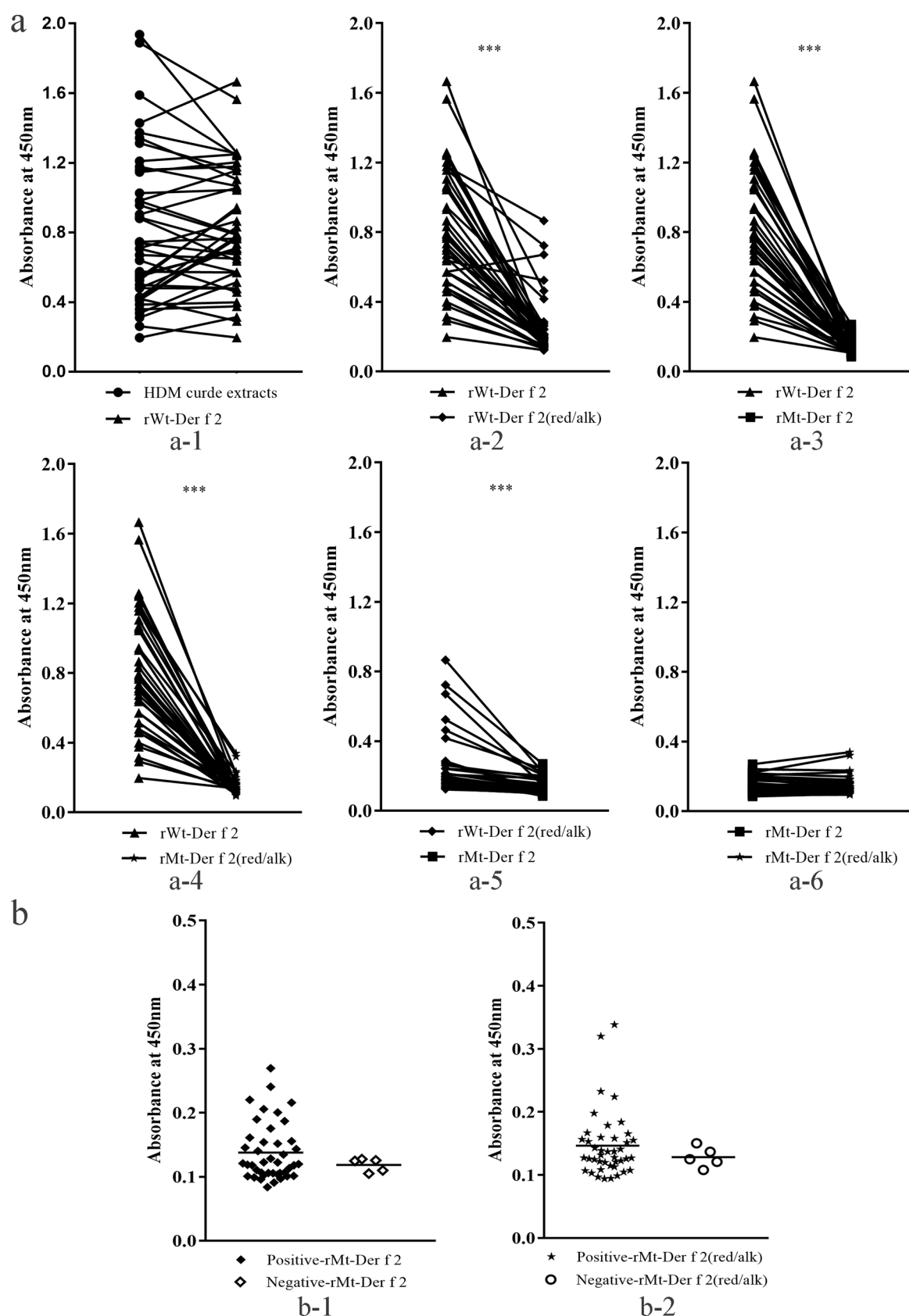


**Fig. 3.** The reactivity of 4 candidates of desensitization vaccines against Der f 2 to IgEs from sera of HDM allergic patients. (a) ELISA. (b) Unpaired *t* test. The IgE-binding activity of rMt-Der f 2 (b-1) and rMt-Der f 2 (red/alk) (b-2) to IgEs from sera of HDM allergic patients was comparable to that of non-allergic individuals (both  $P > 0.05$ ).

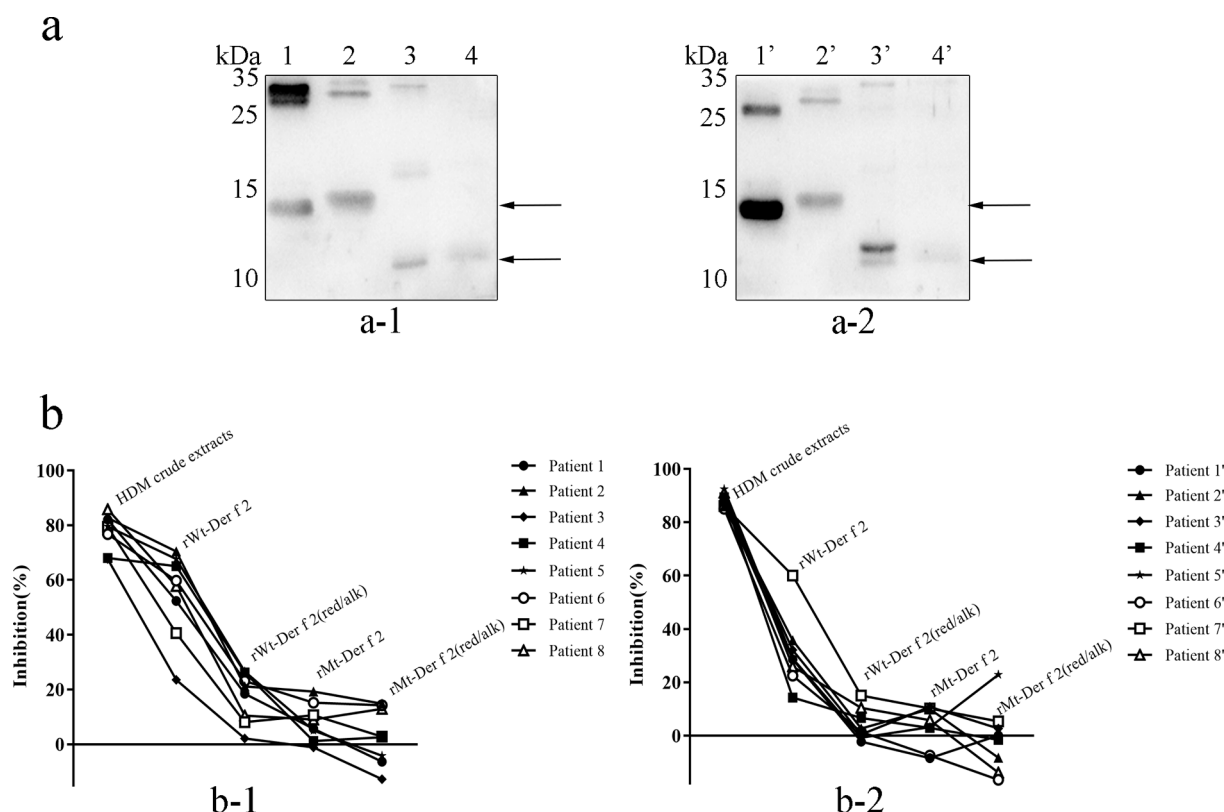


**Fig. 4.** Western blot and inhibition ELISA. (a) Western blot. Lane 1, 2, 3 and 4 - rWt-Der f 2, rWt-Der f 2 (red/alk), rMt-Der f 2 and rMt-Der f 2 (red/alk) at the non-reducing condition respectively (a-1); Line 1', 2', 3' and 4' - rWt-Der f 2, rWt-Der f 2 (red/alk), rMt-Der f 2 and rMt-Der f 2 (red/alk) at the reducing condition respectively (a-2). (b) Inhibition ELISA. The inhibitory effect of rWt-Der f 2, rWt-Der f 2 (red/alk), rMt-Der f 2 and rMt-Der f 2 (red/alk) on HDM crude extracts orderly reduced at inhibitory concentration of 10  $\mu\text{g/mL}$  (b-1) and 20  $\mu\text{g/mL}$  (b-2).

f 2 (red/alk) was possibly explained by destructive conformational IgE-binding B epitopes. Compared with rWt-Der f 2, rMt-Der f 2 developed by deleting nucleotide sequences encoding IgE-binding B epitopes, showed lower IgE-binding activity. Similarly, the decrease of IgE-binding activity of rWt-Der f 2 to rMt-Der f 2 was possibly explained by destructive linear IgE-binding B epitopes. Further, the reactivity of rMt-Der f 2 to IgEs from sera of HDM allergic patients was lower than that of rWt-Der f 2 (red/alk), which indicated that linear rather than conformational IgE-binding B epitopes possibly played a critical role in Der f 2 mediating allergic responses. Then, the reactivity of rMt-Der f 2 (red/alk) with removed linear and conformational IgE-binding B epitopes to IgEs from sera of 42 HDM allergic patients was comparable to that of rMt-Der f 2 with destructive linear IgE-binding B epitopes, which was possibly explained that destructive consecutive amino acid sequences also involved in conformational IgE-binding B epitopes. Summarily, it indicated that both linear and conformational IgE-binding B epitopes were critical for Der f 2 mediating allergic responses. Therefore, the hypoallergenic Der f 2 vaccines developed by only breaking disulfide bonds to destroy conformational IgE-binding B epitopes could remain IgE-binding activity. Accordingly, rMt-Der f 2 or rMt-Der f 2 (red/alk) is of less allergenicity than these hypoallergenic vaccines against Der f 2 developed by only destroying intramolecular disulfide bonds.

On the other hand, T cell epitopes also played a critical role in AIT, which mainly mediated the immune tolerance via generating regulatory T cells and secreting inhibitory cytokines (Jutel et al., 2006, Akdis and Akdis, 2007). Thus, we preserved nucleotide sequences encoding T cell epitopes of Der f 2, which based on the preserved T cell proliferation ability when the amino acid fragment (8–119) of rWt-Der f 2 was destroyed (Ichikawa et al., 1998).

## 5. Conclusion

We developed the candidate of desensitization vaccines against Der f 2, rMt-Der f 2 or rMt-Der f 2 (red/alk), nearly without IgE-binding activity, which indicated that rMt-Der f 2 or rMt-Der f 2 (red/alk) had the potential to prevent HDM allergic patients treated with AIT from allergic adverse effects.

### CRediT authorship contribution statement

**Xiao-Jing Ling:** Writing – original draft, Writing – review & editing, Methodology, Validation, Visualization. **Chen Pan:** Writing – original draft, Writing – review & editing, Methodology, Validation, Visualization. **Ling-Xiao Tan:** Writing – original draft, Writing – review & editing, Methodology, Validation, Visualization. **Ying Zhu:** Writing – original draft, Writing – review & editing, Methodology, Validation, Visualization. **Yu-Xing Yang:** Writing – original draft, Writing – review & editing, Methodology, Validation, Visualization. **Xiao-Fei Zeng:** Software, Visualization. **Jin-Lyu Sun:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision. **Ji-Fu Wei:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision. **Chen Lu:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.imbio.2022.152217>.

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