

Comparison of skin prick test with serially diluted wild-type and genetically engineered recombinant Der f2

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Background: C8/119S, a genetically engineered less allergenic mutant of group II allergen (Der f2) of house dust mite, *Dermatophagoides farinae*, was constructed in order to reduce the risk of anaphylactic reactions of allergen specific immunotherapy.

Objective: To further evaluate, with a larger number of mite-allergic patients, the safety of C8/119S for the treatments of humans.

Method: We tested the dose-dependent responses of 20 mite-allergic volunteers to a skin prick test with C8/119S and wild-type recombinant Der f2 and compared the biologic potentials of these allergens to induce type I allergic reactions. In a separate experiment, we compared IgE binding capacities to C8/119S and to wild-type recombinant Der f2 in individual sera from 34 mite-allergic donors.

Results: The concentration of C8/119S needed to induce positive skin prick test (SPT) reaction was at least 100 times more than that of recombinant Der f2 in 95% of the volunteers tested. Consistent with this result, IgE binding data showed that 85% of the mite-allergic donors had little or no detectable IgE bound to C8/119S. Our data also shows that a minority of mite-allergic patients responded in a similar manner to both C8/119S and wild-type recombinant Der f2.

Conclusion: Our data confirms that C8/119S is much less allergenic and thus can be used safely for immunotherapy of most of mite-allergic patients. Care should still be taken because, in a minority of patients, C8/119S may cause similar type I allergic reactions as does wild-type recombinant Der f2.

Ann Allergy Asthma Immunol 2000;84:366–368.

INTRODUCTION

Although allergen specific immunotherapy is an efficient treatment for allergic diseases to defined allergens,^{1,2} the biggest disadvantage is a risk of anaphylactic reactions as a result of administration of native allergens to allergic patients.³ For improvement of current forms of immunotherapy, recombinant allergen derivatives with reduced anaphylactic potential have recently attracted attention.^{4,5}

Group II allergen (Der f2) of house dust mite, *Dermatophagoides farinae*, is one of the major allergens in various allergic diseases.⁶ A genetically engineered mutant of Der f2, C8/119S, in which the disulfide bond linking the N- and C-terminal sequences of Der f2 was disrupted by replacing cysteine at the 8th and 119th residues with serine, has been constructed recently.⁷ C8/119S has been shown to have little or no binding activity to IgE in sera from mite-allergic patients,^{7,8} little ability to inhibit IgE binding to wild-type recombinant Der f2, and a markedly reduced capacity to induce in vivo skin prick test reactivity and in vitro histamine release from peripheral blood basophils⁷ while retaining the same⁷ or even higher (our unpublished results) capacity to stimulate T-cell proliferation.

C8/119S is therefore potentially very useful for mite-allergen specific immunotherapy with reduced risk of anaphylaxis even at high doses. In fact, C8/119S has been shown to hyposensitize mice⁹ and application of C8/119S to the treatment of allergies in human is now under preparation in our institution. As published data with respect to in vivo and in vitro allergic reactions were based on results with only four allergic volunteers⁷ and thus should be considered preliminary, further evaluation with a larger number of patients was necessary to evaluate the safety of C8/119S for the treatments of humans. For this study, we tested the dose-dependent responses of 20 mite-allergic volunteers to a skin prick test with C8/119S and wild-type recombinant Der f2 and compared the biologic potentials of these allergens to induce type I allergic reactions. In a separate experiment, we compared IgE binding capacities to C8/119S and wild-type recombinant Der f2 in individual sera from 34 mite-allergic donors.

MATERIALS AND METHODS

Materials

Wild-type recombinant Der f2 (rDerf2) and its genetically engineered mutant, C8/119S, were prepared as described previously.⁷

Skin Prick Test (SPT)

Twenty mite-allergic volunteers aged 20 to 25, whose RAST scores for *Dermatophagoides farinae* were more than 3, were skin prick tested as described elsewhere.¹⁰ Solutions of ten times serially diluted wild-type rDer f2 and C8/119S (1 ng/mL to 10 µg/mL) were applied. A flare reaction area of more than 10 mm or wheal reaction area of

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Received for publication August 17, 1999.

Accepted for publication in revised form October 27, 1999.

more than 3 mm was considered a significant SPT reaction.

IgE Binding ELISA

Sera were obtained from 34 mite-allergic donors aged 3 to 42 years. Diluted serum was added to wells of microtiter plates immobilized with 1 $\mu\text{g/mL}$ of either wild-type rDer f2 or C8/119S and incubated. IgE bound to the well was detected with peroxidase-conjugated anti-human IgE (Bio Source International, Inc, Tago Products, Camarillo, CA). The signal was detected with TMB (3,3',5,5'-tetramethyl benzidine) by measuring the absorbance at 450 nm. Titers of IgE were expressed in arbitrary units (AU)/mL on the basis of a laboratory-determined, mite-allergic standard serum.

Statistical Analysis

A software package (Statview, CA) was used for statistical analysis. Difference between the positive SPT rates with rDerf2 and with C8/119S was analyzed by the chi square test. Difference between IgE binding to rDerf2 and to C8/119S was analyzed with the Mann-Whitney U test. *P* values of less than .05 were considered statistically significant.

Informed consent was obtained from all the volunteers and donors.

RESULTS

Skin Prick Tests with Wild-Type rDer f2 and C8/119S

Twenty mite-allergic volunteers whose RAST scores for *Dermatophagoides farinae* were more than 3, were skin prick tested with ten times serially diluted rDer f2 and C8/119S (1 ng/mL to 10 $\mu\text{g/mL}$). Mean diameters of the flare reactions to 10 $\mu\text{g/mL}$ rDer f2 ranged from 10 mm to more than 30 mm as shown in Figure 1b. With 1 $\mu\text{g/mL}$ rDer f2, 15 patients (75%) still showed flare reactions of more than 10 mm (Fig 1a). In contrast, with 1 $\mu\text{g/mL}$ C8/119S, only three patients (15%) showed flare reactions of more than 10 mm (Fig 1c), and even with 10 $\mu\text{g/mL}$ of C8/119S, only six patients (30%) did (Fig 1d). Difference between positive SPT rates (more than 10-mm flare reaction) with 1 $\mu\text{g/mL}$ of rDerf2 and C8/119S was statistically significant (*P* = .020). Minimum concentrations of C8/119S and rDer f2 eliciting wheal reactions more than 3 mm in diameter were obtained and their ratio (C8/119S/rDer f2) was calculated for each patient.

The ratios for 19 volunteers (95%) were more than 100, with 12 volunteers (60%) showing more than 1,000, while it was only 1 for one (5%) volunteer (Fig 2).

IgE Binding to Wild-Type rDer f2 and C8/119S

In a separate experiment, IgE binding capacities to wild-type rDer f2 and C8/119S were compared individually by means of ELISA using sera from 34 mite-allergic donors (Fig 3). The IgE binding capacity to wild-type rDer f2 varied, and the mean titer was 68.7 AU/mL. In eight (23.5%) patients, it was more than 100 AU/mL. In contrast, the IgE binding capacity to C8/119S was significantly lower (mean titer; 3.9 AU/mL) (*P* < .0001) and in 29 (85.3%) donors, there was no significant binding (titer less than 5). Interestingly, five donors (14.7%) who showed detectable IgE binding to C8/119S also showed much higher IgE binding to wild-type rDer f2 (titer more than 100) than other donors.

DISCUSSION

In this study, we compared the biologic potential of C8/119S and wild-type rDer f2 to induce type I allergic reactions by comparing the dose response of SPT reactions. The concentration of C8/119S needed to induce positive SPT reaction was at least 100 times more than that of rDer f2 in 95% of the volunteers tested, suggesting that C8/119S is at least 100 times less capable than wild-type rDer f2 of pro-

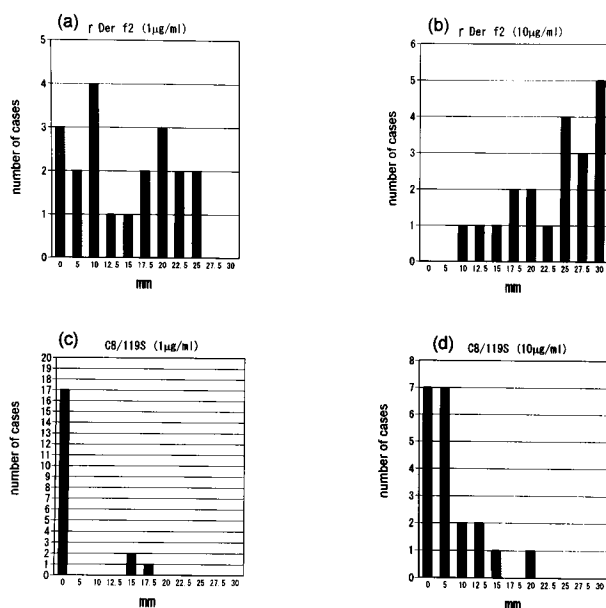


Figure 1. Distribution of flare diameters in response to prick tests with wild-type rDer f2 and C8/119S.

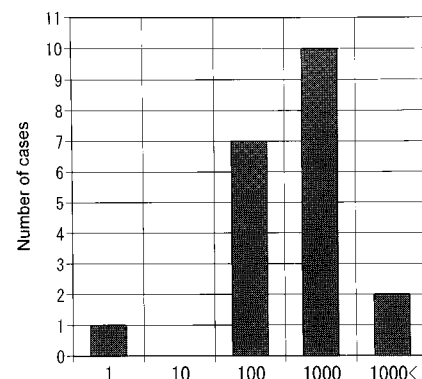


Figure 2. Distribution of the threshold ratio (C8/119S/rDer f2) for skin prick test.

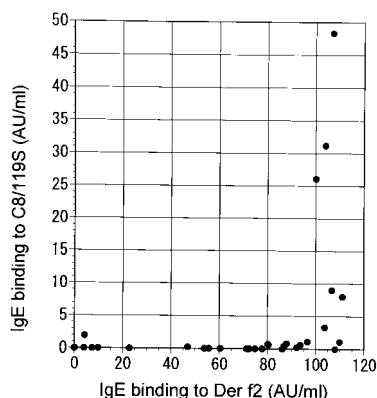


Figure 3. IgE binding to wild-type rDer f2 and C8/119S. IgE binding capacities to wild-type rDer f2 and C8/119S were compared individually by means of ELISA using sera from 34 mite-allergic donors as described in "Materials and Methods."

voking type I allergic reactions in most mite-allergic patients. Consistent with this result, IgE binding data showed that 85% of mite-allergic donors had little or no detectable IgE bound to C8/119S.

Our data also shows that a minority of mite-allergic patients responded to both C8/119S and wild-type rDer f2 in a similar manner. Threshold levels of C8/119S and wild-type rDer f2 for positive SPT reactions were the same in one out of 20 (5%) volunteers, and five out of 34 donors (14.7%) had detectable levels of C8/119-specific IgE. One interesting finding in our data is that those five donors also showed significantly higher levels of wild-type rDer f2-specific IgE than other patients. A possible explanation is that IgE specific to wild-type rDer f2 in-

cludes a subgroup which recognizes conformational epitopes possibly conserved in C8/119S, and that such a subgroup becomes detectable when the IgE level of wild-type rDer f2 is significantly higher. Alternatively, as the sequential epitopes are almost the same for C8/119S and wild-type rDer f2, IgE from these patients might recognize the sequential epitopes of rDer f2.

Overall, our data confirms that C8/119S is much less allergenic for mite allergic patients by showing that C8/119S, in a dose dependent manner, is at least 100 times less capable than wild-type Der f2 of causing positive SPT reactions. C8/119S thus may be able to be used safely as a tool for allergen specific immunotherapy for most mite-allergic patients. Care, however, must be taken since C8/119S may cause similar type I allergic reactions as does wild-type Der f2 in a minority of patients. Those with higher titers of IgE to wild-type Der f2 might be at higher risk for such an adverse reaction, as those patients tend to have detectable levels of IgE to C8/119S.

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