

Engineering of the major house dust mite allergen Der f 2 for allergen-specific immunotherapy

Toshiro Takai*, Toyokazu Yokota, Masaaki Yasue, Chiharu Nishiyama, Toshifumi Yuuki, Akio Mori¹, Hirokazu Okudaira¹, and Yasushi Okumura

Bioscience Research and Development Laboratory, Asahi Breweries, Ltd., 1-1-21 Midori, Moriya-cho, Kita-sohma-gun, Ibaraki 302-01, Japan.

¹Department of Medicine and Physical Therapy, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.

*Corresponding author (e-mail: toshihiro.takai@asahibeer.co.jp).

Received 31 March 1997; accepted 4 June 1997

A major problem with allergen-specific immunotherapy involving repeated injections of allergens is the risk of an anaphylactic reaction. We engineered the major house dust mite allergen, Der f 2, to reduce its capacity to induce skin test reactivity and histamine release from peripheral blood basophils in allergic patients. The engineered allergen, in which the disulfide bond that linked the N- and C-terminal sequences of Der f 2 was disrupted, retained T-cell epitopes essential for immunotherapy and ability to stimulate T-cell proliferation. Such engineered allergens are potentially useful for safer and more effective immunotherapy for allergies.

Keywords: allergen engineering, protein design, T-cell proliferation, immunotherapy

More than 20% of the world's population suffers from IgE-mediated type I allergic symptoms. Allergens are proteins characterized by their ability to induce a pathogenic IgE response in susceptible individuals^{1,2}, giving rise to asthma, rhinitis, and atopic dermatitis. Allergen-specific immunotherapy involves repeated injections of allergens over prolonged periods and has been performed since the early 1900s³. Clinical trials of allergen-specific immunotherapy using short peptides containing T-cell epitopes have been shown to induce unresponsiveness of T cells or a change in the pattern of cytokines produced by CD4⁺ T cells⁴⁻⁶. Peptides can have the advantage of not being able to cross-link the complex of the allergen-specific IgE and the IgE receptor (FcεRI) on the surface of mast cells and basophils—which would induce the release of mediators—because of an absence or reduction in the number of IgE epitopes⁷⁻⁹. Therefore, peptide-based immunotherapy has the advantage of reduced risk of anaphylactic reaction over conventional allergen-specific immunotherapy. The drawback of peptide-based immunotherapy is the large diversity in T-cell epitopes due to the diversity of major histocompatibility complex (MHC) and T-cell receptor (TCR) among individuals^{6,10-13}.

House dust mite (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*) allergens are major causative factors associated with various allergic diseases⁴⁻¹⁶, and the major allergens are classified as group 1 (Der f 1 and Der p 1, Mr 25,000) or group 2 (Der f 2 and Der p 2, Mr 14,000)¹⁷⁻¹⁹. The majority of sera from patients allergic to house dust mites have binding activities to both group 1 and 2 allergens. Genetic linkage between specific IgE responses to these allergens and genes on human chromosomes has been intensively investigated^{1,2}. Analysis of B-cell^{7,20-23} and T-cell epitopes^{13,24-26} of the major allergens also has been carried out to clarify their relationships to immunogenicity and to apply such epitope moieties to immunotherapy^{4,6,27}.

cDNA clones for Der f 2, the group 2 allergen of *D. farinae*^{28,29}, encode proteins consisting of 129 amino-acid residues with

marked homology to Der p 2, the group 2 allergen of *D. pteronyssinus*. It has been suggested that IgE epitopes of Der f 2 and Der p 2 are dependent on the integrity of the conformational structure of the allergens rather than the contiguous sequence of amino acids^{7,23}. We determined the location of three disulfide bonds of native Der f 2 (Cys8-Cys119, Cys21-Cys27, and Cys73-Cys78)³⁰ and assumed that some of them played critical roles in expressing IgE-binding capacity.

We show that two of the three intramolecular disulfide bonds involved in the maintenance of the antigenic structure of Der f 2 were required for recognition by IgE in allergic patient sera by analyzing nine cysteine mutants of Der f 2 expressed in *Escherichia coli*. One of the mutants, C8/119S, in which the disulfide bond that linked the N- and C-terminal sequences of Der f 2 was disrupted, had a markedly reduced capacity to induce in vivo skin test reactivity and in vitro histamine release from peripheral blood basophils in mite-allergic patients. However, C8/119S retains complete activity to stimulate T-cell proliferation. Thus, C8/119S is potentially useful for immunotherapy with a reduced risk of anaphylaxis even at high dose^{26,31-33}. Engineered allergens with reduced reactivity to IgE and composed of almost the entire amino-acid sequence of the native allergen, including most of the T-cell epitopes essential for immunotherapy, might be useful for the treatment of allergies.

Results

Reactivities of cysteine mutants of Der f 2 to IgE in allergic patient sera. We generated nine variants of Der f 2, six with a mutation of each of six cysteines to a serine and three with double mutations of each of the pairs of cysteines forming the disulfide bonds to a pair of serines (Fig. 1A). The reactivities of IgE in allergic patient sera to the wild-type and the mutant Der f 2s expressed in *E. coli* were examined by immunoblot analysis. Sera from all ten patients tested showed similar IgE-binding patterns. The mutants C8S, C119S, and C8/119S showed no or markedly low IgE binding.

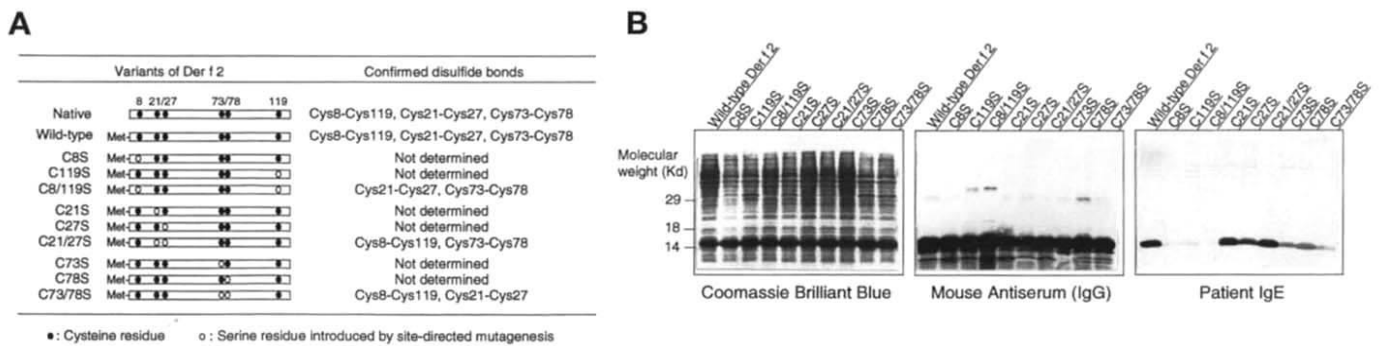


Figure 1. (A) Schematic representation of variants of Der f 2. Native Der f 2 purified from mite extract and the wild-type and nine cysteine mutants of Der f 2 expressed in *E. coli* with a methionine at the first N-terminal residue are shown. (B) Expression of the wild-type and nine cysteine mutants of Der f 2 and their binding to IgE from an allergic patient analyzed by immunoblotting.

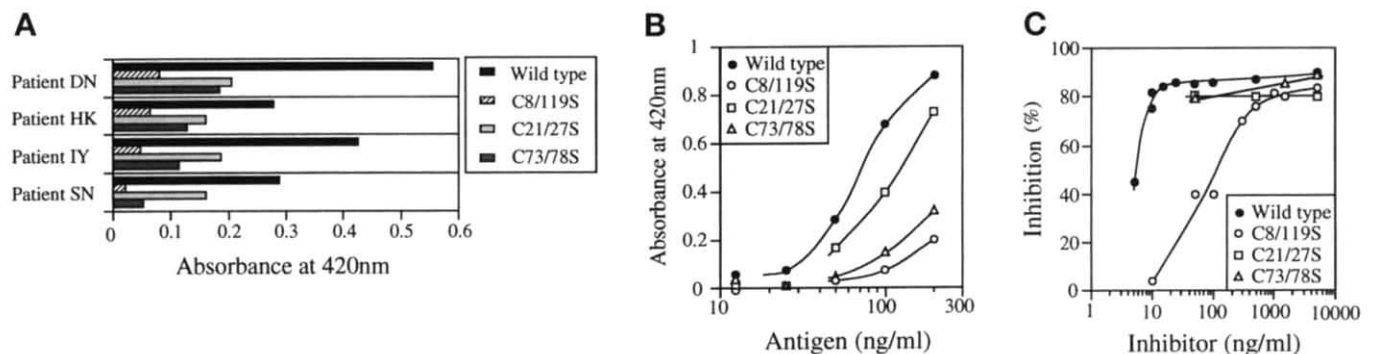


Figure 2. RAST-EIA and inhibition assay using purified wild-type and three cysteine double-mutants of Der f 2. (A) IgE-binding profiles of four patients allergic to house dust mite. Initials of the patients' names are indicated (DN, HK, IY, and SN). The concentration of antigens in the immobilization step was 50 ng/ml. (B) IgE-binding profile of patient SN. (C) Inhibition of IgE binding to the immobilized wild-type Der f 2 by addition of Der f 2 variants. The sera from patient SN was used.

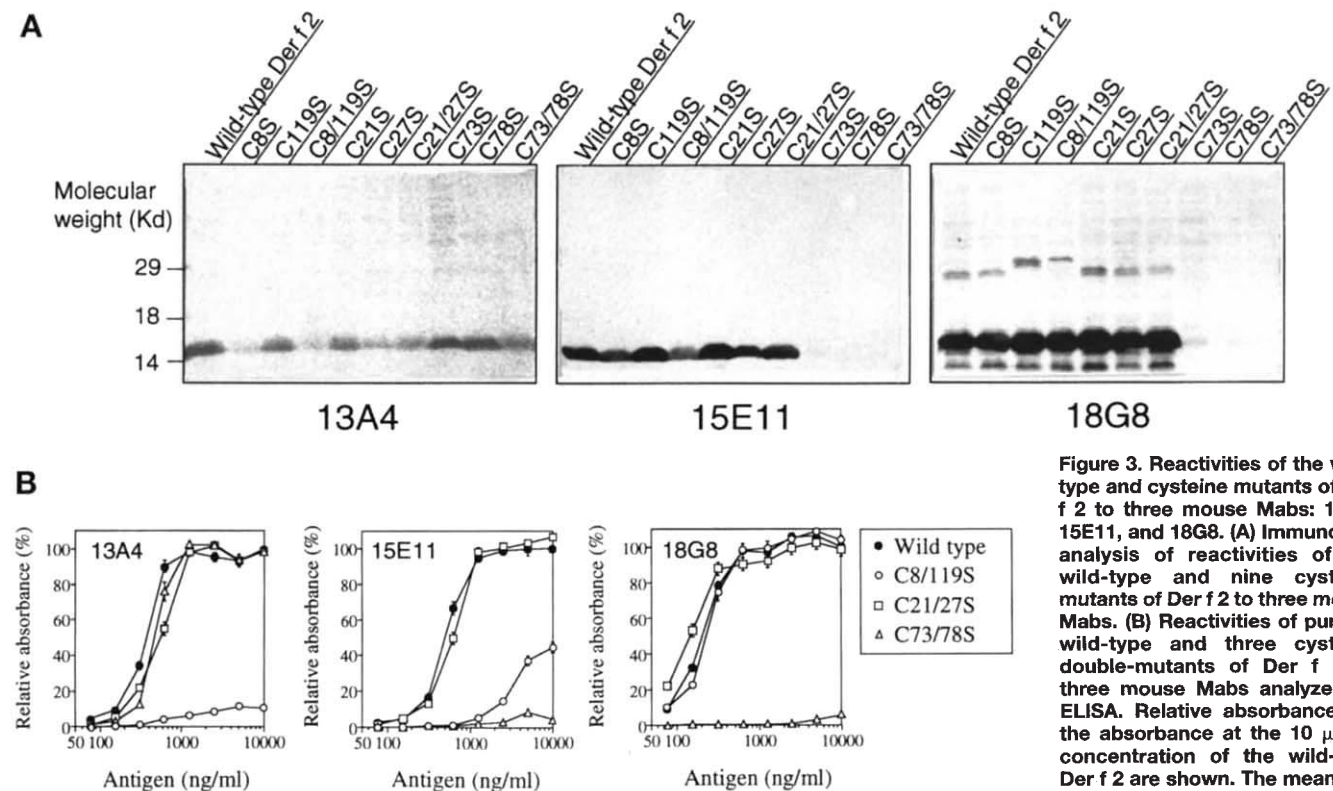


Figure 3. Reactivities of the wild-type and cysteine mutants of Der f 2 to three mouse Mabs: 13A4, 15E11, and 18G8. (A) Immunoblot analysis of reactivities of the wild-type and nine cysteine mutants of Der f 2 to three mouse Mabs. (B) Reactivities of purified wild-type and three cysteine double-mutants of Der f 2 to three mouse Mabs analyzed by ELISA. Relative absorbances to the absorbance at the 10 μ g/ml concentration of the wild-type Der f 2 are shown. The mean and standard error of the mean of triplicate points are shown.

RESEARCH

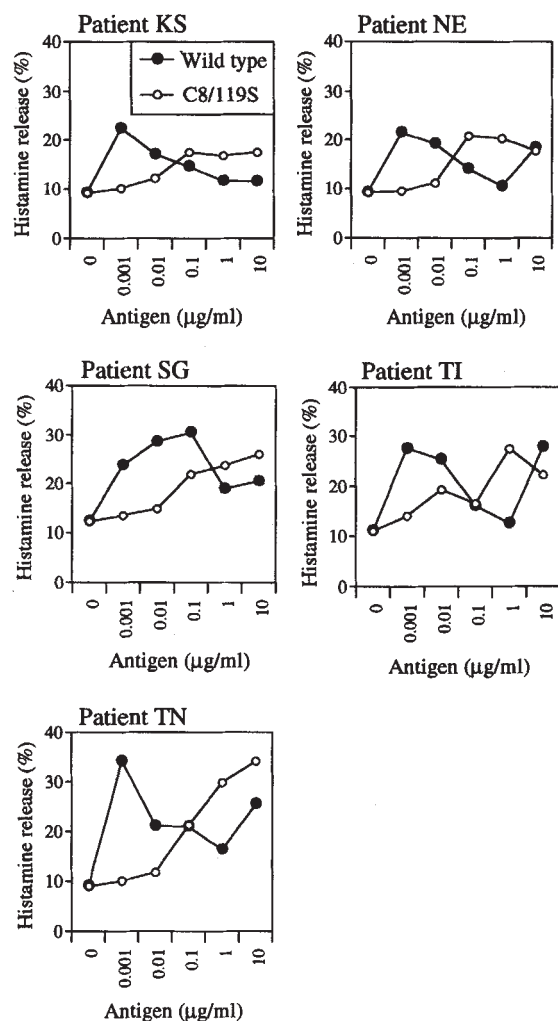


Figure 4. Histamine release from basophils stimulated with the wild-type Der f 2 and its cysteine double-mutant, C8/119S. Peripheral blood leukocytes were obtained from mite-allergic patients whose initials are indicated (KS, NE, SG, TI, and TN).

The mutants C73S, C78S, and C73/78S showed moderately reduced IgE binding. The mutants C21S, C27S, and C21/27S showed no or little reduction of IgE binding (Fig. 1B).

Three cysteine double-mutants, C8/119S, C73/78S, and C21/27S, were selected for detailed experiments using purified recombinant proteins because cysteine single-mutants potentially cause incorrect intramolecular disulfide bonds, dimerization or aggregation of the protein in solution. The correct disulfide bond formations of the purified wild-type and three cysteine double-mutants were confirmed by peptide mapping³⁰.

The IgE-binding capacities of the refolded recombinant proteins with correct disulfide bonds were determined by radioallergosorbent test-enzyme immunoassay (RAST-EIA)²¹. All patients tested showed similar profiles of IgE-binding capacity: marked reduction in the mutant C8/119S compared with the wild-type, moderate reduction in C73/78S, and significant but not remarkable reduction in C21/27S (Fig. 2A and B). The capacities of the mutant Der f 2s to inhibit IgE binding to the wild-type Der f 2 were also examined using serum from patient SN by inhibition assay (Fig. 2C). IgE binding to the wild-type Der f 2 was almost completely inhibited by addition of an excess amount of each cysteine double-mutant of Der f 2. On the other hand, an approxi-

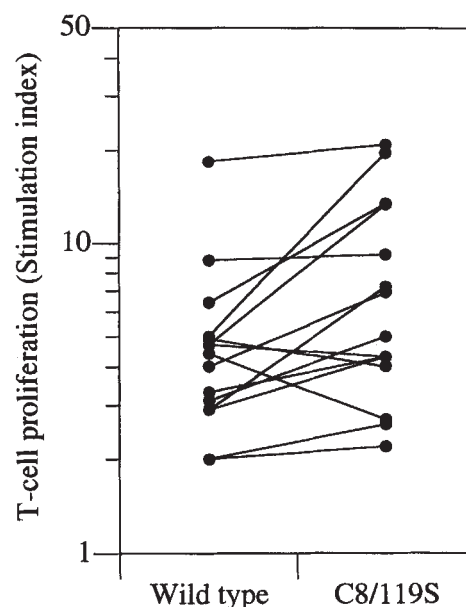


Figure 5. Proliferative response of T cells stimulated with the wild-type Der f 2 and its cysteine double-mutant, C8/119S. Peripheral blood mononuclear cells were obtained from 16 mite-allergic patients. Results are presented as stimulation indices that were calculated as the ratio of the counts/min of culture wells containing allergen with that of wells containing medium alone. The mean of four points for each patient is shown.

mately 100-fold increase in the concentration of the mutant C8/119S was required to achieve the levels of inhibition obtained with the wild-type Der f 2.

Reactivities of cysteine mutants of Der f 2 to anti-Der f 2 monoclonal antibodies (Mabs). Epitope analysis of Der f 2 for mouse anti-Der f 2 Mabs is important for understanding the antigenic structure of Der f 2 recognized by polyclonal human IgE. Therefore, the reactivities of the wild-type and cysteine mutants of Der f 2 to three mouse Mabs³⁴ were examined by immunoblot analysis and enzyme-linked immunosorbent assay (ELISA) (Fig. 3). The reactivity to the Mab, 13A4, was markedly reduced in the mutants C8S and C8/119S. The reactivity to 15E11 was markedly reduced in C8/119S, C73S, C78S, and C73/78S. The reactivity to 18G8 was markedly reduced in C73S, C78S, and C73/78S.

Activity of C8/119S to induce histamine release from basophils from allergic patients. Peripheral blood basophils from five allergic patients were stimulated with the wild-type Der f 2 or C8/119S, and histamine release was measured (Fig. 4). The threshold concentration of C8/119S (approximately 0.1 µg/ml) to stimulate peripheral basophils to release histamine was about 100 times more than that of the wild-type Der f 2 (approximately 0.001 µg/ml).

Activity of C8/119S to stimulate proliferation of T cells from allergic patients. Proliferation of T cells in peripheral blood mononuclear cells from 16 mite-allergic patients stimulated with the wild-type Der f 2 or C8/119S was assayed. The proliferation of polyclonal T cells stimulated with C8/119S was the same as, or higher than, that stimulated with wild-type Der f 2 (Fig. 5).

Activities of cysteine mutants of Der f 2 to induce urticarial type I reactions in allergic patients. The capacity of the wild-type and three cysteine double-mutants to elicit cutaneous reactions was evaluated in three volunteers by *in vivo* skin prick test³⁵. Using the wild-type Der f 2, C21/27S, or C73/78S, typical urticarial type I reactions were observed, while C8/119S skin prick tests were negative (Fig. 6).

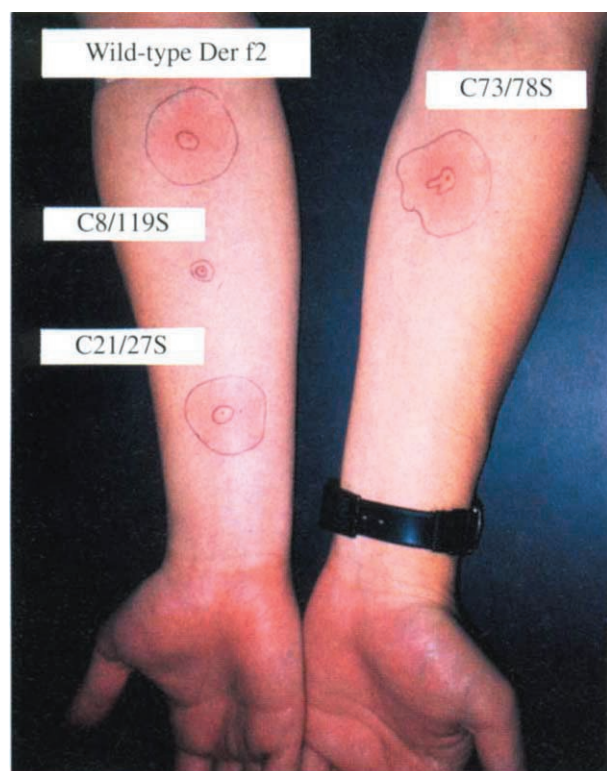


Figure 6. Skin prick test with the recombinant Der f 2 and its three cysteine double mutants, C8/119S, C21/27S, and C73/78S.

Discussion

The structure of the wild-type Der f 2 is maintained by three rigid disulfide bonds²⁰. We concluded that Cys8-Cys119 is the most essential of the three disulfide bonds for maintaining the Der f 2 structure required to bind human IgE, followed by the Cys73-Cys78 disulfide bond. The result shown in Fig. 2C suggests that every cysteine double-mutant of Der f 2 has almost all the IgE epitopes, but the total affinity of IgE epitopes in the mutant C8/119S was markedly reduced compared with that in the wild-type Der f 2. These findings on IgE binding correlate well with data obtained using other variants of Der f 2 to which a deletion or point mutation was introduced. This suggested an important contribution of the residues around the disulfide bond Cys8-Cys119, and a significant contribution of the residues around Cys73-Cys78, for maintaining the IgE binding structure of Der f 2 (refs. 21,23).

The mAb binding data presented in Figure 3 further supports our conclusion. Cys73 and Cys78 are included in the sequence (67–90) of Der f 2 that contains the core of the epitopes for the mAbs²³. Further epitope analysis for the monoclonal Abs will provide useful insights into the polyclonal IgE epitopes in patient sera of Der f 2.

Der f 2 is homologous to Der p 2 from the dust mite species *D. pteronyssinus*²³. Smith and Chapman²² reported on the IgE reactivity of cysteine variants of Der p 2. Using a glutathione-S-transferase fusion protein system, they showed that two mutant Der p 2 molecules, C8G and C119Y, in which a single mutation had been introduced at each of the two cysteine residues involved in the disulfide bond of Cys8-Cys119, maintain the same reactivity to IgE as the wild type Der p 2. On the other hand, we used recombinant Der f 2 variants with an additional methionine at the first N-terminal residue and confirmed the correct disulfide bond formation in the three cysteine double-mutants. The discrepancy between our findings and theirs might result from dif-

ferences between either Der f 2 and Der p 2, the patients, the amino acids substituted for cysteine, or the preparation of recombinant products.

Allergen-specific immunotherapy involves the stepwise subcutaneous injection of increasing concentrations of specific allergens to which a patient is sensitive to achieve a state of immunological tolerance³⁶. Secrist et al.³¹ reported that CD4⁺ T cells from allergic donors produced little interleukin 4 (IL-4) when stimulated with high concentrations of allergen, although they produced high levels of IL-4 when stimulated with low concentrations of allergen. Their findings suggest that, ideally, allergen should be administered at a higher dose to change the Th1/Th2 bias³⁷. This may, however, cause an anaphylactic reaction, induced by the release of mediators from mast cells and basophils stimulated by aggregation of the complex of allergen-specific IgE and FcεRI on the cell surface cross-linked with allergen^{8,9}. The variant of the major house dust-mite allergen Der f 2, C8/119S had a much lower affinity to IgE in patient sera. Furthermore, peripheral basophils and skin mast cells do not degranulate in the presence of C8/119S in allergic patients (Figs. 4 and 6). Therefore, allergen-specific immunotherapy with C8/119S should not cause anaphylaxis even at relatively high doses.

Clinical trials of allergen-specific immunotherapy using short-lengthened peptides containing T-cell epitopes of allergens have been carried out⁴⁵. As with immunotherapy using a modified allergen such as C8/119S, the peptide-based immunotherapy has the advantage of less IgE binding⁷. A problem associated with peptide-based immunotherapy, however, is a diversity of T-cell epitopes due to the diversity of MHC class II molecules and TCRs in humans^{6,10–13}. Immunotherapy with engineered allergens such as C8/119S, which retain most of the T-cell epitopes, would solve this problem. We observed that the proliferation of polyclonal T cells (Fig. 5) reactive to Der f 2 from mite-allergic patients stimulated with C8/119S was the same as, or even higher than, that stimulated with wild-type Der f 2. The higher activities of C8/119S to stimulate patient T cells suggest that C8/119S is processed more efficiently in antigen-presenting cells than the wild-type Der f 2 as a result of disruption of the disulfide bond. Therefore, the cysteine double-mutant of Der f 2, C8/119S, is potentially useful for immunotherapy specific for house dust mites in that it not only reduces the risk of anaphylaxis, but it also maintains most of the T-cell epitopes.

Experimental protocol

Expression of the wild-type and cysteine mutants of Der f 2. The wild-type and nine cysteine mutants of Der f 2 (C8S, C119S, C8/119S, C21S, C27S, C21/27S, C73S, C78S, and C73/78S) were expressed in *E. coli* BL21 (ref. 21) transformed with expression vectors constructed by site-directed mutagenesis.

Immunoblot analysis. The method was as described previously²³. Briefly, total cellular proteins expressed in *E. coli* BL21 transformed with expression vectors of the wild-type and nine cysteine mutants of Der f 2 were subjected to sodium dodecyl sulfate polyacrylamide electrophoresis (18% acrylamide) and electroblotted. The membranes were stained with Coomassie brilliant blue, probed with a combination of mouse anti-Der f 2 antiserum (or mouse Mabs) and horseradish peroxidase-conjugated anti-mouse IgG antibodies (Biorad, Richmond, CA), or that of a mite-sensitive human allergic serum and horse radish peroxidase-conjugated anti-human IgE (ε-specific) antibodies (Cappel, Malvern, PA).

Purification of the wild-type and cysteine mutants of Der f 2. The wild-type and three cysteine double-mutants of Der f 2 (C8/119S, C21/27S, and C73/78S) produced as inclusion bodies in *E. coli* BL21 were recovered, solubilized with buffer containing urea, refolded by dialysis against buffer, and purified by anion exchange column chromatography using CM-Sepharose (Pharmacia, Uppsala, Sweden) or DEAE-Toyopearl (Tosoh, Tokyo, Japan) as described²¹. The correct disulfide bond formation of the purified recombinant Der f 2 and its cysteine double-mutants was confirmed by peptide mapping as described³⁰.

RESEARCH

RAST-EIA. IgE reactivities of the purified wild-type Der f 2 and its cysteine double-mutants were measured by RAST-EIA using a RAST-EIA kit (Pharmacia) as previously described²¹. Briefly, serum of each allergic patient was incubated with paper discs coupled with each of the purified wild-type Der f 2 and its cysteine double-mutants in various amounts. Then, the IgE that bound to the disc was detected with β -galactosidase-conjugated anti-human IgE antibodies by measuring the absorbance at 420 nm.

Inhibition assay. The method was as described²¹. Briefly, the serum of each allergic patient was incubated with various concentrations of each of the purified wild-type Der f 2 and its cysteine double-mutants, and then the solution was added to a paper disc presoaked in wild-type Der f 2. The IgE that bound to discs was detected with β -galactosidase-conjugated anti-human IgE antibodies.

ELISA. The method was as described³⁸. Briefly, each solution containing mouse Mabs was added into wells of micro-titer plates immobilized with each of the purified wild-type Der f 2 and its cysteine double-mutants in various amounts and incubated. Then, Mab that bound to the well was detected with biotinylated anti-mouse IgG antibodies and alkaline phosphatase-conjugated avidin by measuring the absorbance at 405 nm.

Histamine release. The leukocytes used for histamine release assays were obtained from peripheral blood of allergic volunteers³⁹. The leukocytes were separated from heparinized blood by dextran sedimentation, washed with tyrode buffer 3 times, and incubated with serially diluted antigens at 37°C for 40 min. Cell-free supernatants and whole cells solubilized with 0.1% Triton X 100 were collected and analyzed for histamine content using a Histamine Release Test Kit (Miles, Spokane, WA). The percentage of released histamine was calculated as the relative amount of histamine in the supernatant to the total of supernatant and cellular histamine.

T-cell proliferation assays. T cells from peripheral blood mononuclear cells from mite-allergic volunteers were cultured in 96-well round-bottomed culture plates with the wild-type Der f 2 or C8/119S (10 μ g/ml), and [³H]-thymidine incorporation was measured as described^{40,41}.

Skin prick test. Skin prick test was performed as described³⁵. Solutions of the wild-type and three cysteine double-mutants of Der f 2 (20 ng/20 μ l) were applied.

Acknowledgments

We thank Yukiko Usui and Minako Fukada, Asahi Breweries, Ltd., for technical assistance, and Midori Akagawa, Asahi Breweries, Ltd., for preparation of the Mabs.

- Moffatt, M.F., Hill, M.R., Cornélis, F., Schou, C., Faux, J.A., Young, R.P., et al. 1994. Genetic linkage of T-cell receptor α/δ complex to specific IgE responses. *Lancet* **343**:1597–1600.
- Marsh, D.G., Neely, J.D., Breazeale, D.R., Ghosh, B., Freidhoff L.R., Ehrlich-Kautzky, E., et al. 1994. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* **264**:1152–1156.
- Noon, L. 1911. Prophylactic inoculation against hay fever. *Lancet* **i**:1572–1573.
- Hoynes, G.F., Kristensen, N.M., Yssel, H., and Lamb, J.R. 1995. Peptide modulation of allergen-specific immune response. *Curr. Opin. Immunol.* **7**:757–761.
- Norman, P.S., Ohman, J.L. Jr., Long, A.A., Creticos, P.S., Geffer, M.A., Shaked, Z., et al. 1996. Treatment of cat allergy with T-cell reactive peptides. *Am. J. Respir. Crit. Care. Med.* **154**:1623–1628.
- van Neerven, R.J.J., Ebner, C., Yssel, H., Kapsenberg, M.L., and Lamb, J.R. 1996. T-cell responses to allergens: epitope-specificity and clinical relevance. *Immunol. Today* **17**:526–532.
- van't Hof, W., Dridijk, P.C., van den Berg, M., Beck-Sicking, A.G., Jung, G., and Aalberse, R.C. 1991. Epitope mapping of the *Dermatophagoides pteronyssinus* house dust mite major allergen Der p II using overlapping synthetic peptides. *Mol. Immunol.* **28**:1225–1232.
- Ishizaka T. and Ishizaka, K. 1984. Activation of mast cells for mediator release through IgE. *Prog. Allergy* **34**:188–235.
- Metzger H., Alcaraz G., Hohman R., Kinet J-P., Pribluda V., and Quarto R. 1986. The receptor with high affinity for immunoglobulin E. *Annu. Rev. Immunol.* **4**:419–470.
- WHO nomenclature committee for factors of the HLA system. 1995. Nomenclature for factors of the HLA system 1995. *Vox Sang.* **69**:359–372.
- Davis, M.M. 1990. T cell receptor gene diversity and selection. *Annu. Rev. Biochem.* **59**:475–496.
- O'Brien, R.M., Thomas, W.R., Nicholson, I., Lamb, J.R., and Tait, B.D. 1995. An immunogenic analysis of the T-cell recognition of the major house dust mite allergen Der p 2: Identification of high and low-responder HLA-DQ alleles and localization of T-cell epitopes. *Immunology* **86**:176–182.
- Yssel, H., Johnson, K.E., Schneider, P.V., Wideman, J., Terr, A., Kastelein, R., and de Vries, J.E. 1992. T cell activation-inducing epitopes of the house dust mite allergen Der p I. Proliferation and lymphokine production patterns by Der p I-specific CD4⁺ T cell clones. *J. Immunol.* **148**:738–745.
- Platts-Mills, T.A.E. and Weck, A.L. 1989. Dust mite allergens and asthma—a worldwide problem. *J. Allergy Clin. Immunol.* **83**:416–427.
- Voohorst, R., Spijksma-Boezeman, M.I.A., Spijksma, F.T.M., Vrekamp, H., Leupen, M.J., and Lyklema, A.W. 1967. The house dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces. Identity with the house dust allergen. *J. Allergy* **39**:325–339.
- Miyamoto, T., Oshima, S., Ishizaki, T., and Sano, S. 1968. Allergic identity between the common floor mite (*Dermatophagoides farinae* Hughes 1961) and house dust as a causative agent in bronchial asthma. *J. Allergy* **42**:14–28.
- Platts-Mills, T.A.E. and Chapman, M.D. 1987. Dust mites: Immunology, allergic disease, and environmental control. *J. Allergy Clin. Immunol.* **80**:755–775.
- Haida, M., Okudaira, H., Ogita, T., Ito, K., Miyamoto, T., Nakajima, T., and Hongo, I. 1985. Allergens of the house dust mite *Dermatophagoides farinae*—Immunochemical studies of four allergic fractions. *J. Allergy Clin. Immunol.* **75**:686–692.
- Yamashita, N., Haida, M., Suko, M., Okudaira, H., and Miyamoto, T. 1989. Allergens of house dust mite *Dermatophagoides farinae*. II. Immunological characterization of four allergic molecules. *Int. Arch. Allergy Appl. Immunol.* **88**:173–175.
- Lombardero, M., Heymann, P.W., Platts-Mills, T.A.E., Fox, J.W., and Chapman, M.D. 1990. Conformational stability of B cell epitopes on group I and group II *Dermatophagoides* spp. allergens. Effect of thermal and chemical denaturation on the binding of mouse IgG and human IgE antibodies. *J. Immunol.* **144**:1353–1360.
- Nishiyama, C., Fukada, M., Usui, Y., Iwamoto, N., Yuuki, T., Okumura, Y., and Okudaira, H. 1995. Analysis of the IgE-epitope of Der f 2, a major mite allergen, by in vitro mutagenesis. *Mol. Immunol.* **32**:1021–1029.
- Smith, A.M. and Chapman, M.D. 1996. Reduction in IgE binding to allergen variants generated by site-directed mutagenesis: contribution of disulfide bonds to the antigenic structure of the major house dust mite allergen Der p 2. *Mol. Immunol.* **33**:399–405.
- Takai, T., Yuuki, T., Okumura, Y., Mori, A., and Okudaira, H. 1997. Determination of the N- and C-terminal sequences required to bind human IgE of the major house dust mite allergen Der f 2 and epitope mapping for monoclonal antibodies. *Mol. Immunol.* In press.
- van Neerven, R.J.J., van't Hof, W., Ringrose J.H., Jansen H.M., Aalberse R.C., Wierenga E.A., and Kapsenberg M.L. 1993. T cell epitopes of house dust mite allergen Der p II. *J. Immunol.* **151**:2326–2335.
- O'Hehir, R.E., Verhoef, A., Panagiotopoulou, E., Keswani, S., Hayball, J.D., Thomas, W.R., and Lamb J.R. 1993. Analysis of human T cell responses to the group II allergen of *Dermatophagoides* species: localization of major antigenic sites. *J. Allergy Clin. Immunol.* **92**:105–113.
- Tsitoura, D.C., Verhoef, A., Gelder, C.M., O'Hehir, R.E., and Lamb, J.R. 1996. Altered T cell ligands derived from a major house dust mite allergen enhance IFN γ but not IL-4 production by human CD4⁺ T cells. *J. Immunol.* **157**:2160–2165.
- Valenta, R. and Kraft, D. 1995. Recombinant allergens for diagnosis and therapy of allergic diseases. *Curr. Opin. Immunol.* **7**:751–756.
- Yuuki, T., Okumura, Y., Ando, T., Yamakawa, H., Suko, M., Haida, M., and Okudaira, H. 1990. Cloning and sequencing of cDNAs corresponding to mite major allergen Der f II. *Jpn. J. Allergol.* **39**:557–561.
- Yuuki, T., Okumura, Y., Ando, T., Yamakawa, H., Suko, M., Haida, M., and Okudaira, H. 1991. Cloning and expression of cDNA coding for the major house dust mite allergen Der f II in *Escherichia coli*. *Agric. Biol. Chem.* **55**:1233–1238.
- Nishiyama, C., Yuuki, T., Takai, T., Okumura, Y., and Okudaira, H. 1993. Determination of three disulfide bonds in a major house dust mite allergen, Der f II. *Int. Arch. Allergy Immunol.* **101**:159–166.
- Secrist, H., DeKruyff, R.H., and Umets, D.T. 1995. Interleukin 4 production by CD4⁺ T cells from allergic individuals is modulated by antigen concentration and antigen-presenting cell type. *J. Exp. Med.* **181**:1081–1089.
- Suko, M., Mori, A., Ito, K., and Okudaira, H. 1995. Oral immunotherapy may induce T cell anergy. *Int. Arch. Allergy Immunol.* **107**:278–281.
- O'Brien, R.M., Byron, K.A., Varigos, G.A., and Thomas, W.R. 1997. House dust mite immunotherapy results in decrease in Der p 2-specific IFN γ and IL-4 expression by circulating T lymphocytes. *Clin. Exp. Allergy* **27**:46–51.
- Akagawa, M., Mori, T., Ando, T., and Okudaira, H. 1992. Specific measurement of a major mite allergen, by an enzyme-linked immunosorbent assay system using monoclonal anti-Der f II antibodies. *Biosci. Biotech. Biochem.* **56**:1725–1727.
- Dredog, S. 1985. Skin prick test. *Allergy* **40**:55–59.
- Norman, P.S. 1993. Modern concepts of immunotherapy. *Curr. Opin. Immunol.* **5**:968–973.
- Parronchi, P., Macchia, D., Piccinni, M.-P., Biswas, P., Simonelli, C., Maggi, E., et al. 1991. Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. *Proc. Natl. Acad. Sci. USA* **88**:4538–4542.
- Nishiyama, C., Yuuki, T., Usui, Y., Iwamoto, N., Okumura, Y., and Okudaira, H. 1994. Effects of amino acid variations in recombinant Der f II on its human IgE and mouse IgG recognition. *Int. Arch. Allergy Immunol.* **105**:62–69.
- Pruzansky, J.J., Grammer, L.C., Patterson, R., and Roberts, M. 1983. Dissociation of IgE from receptors on human basophils: I. Enhanced passive immunization for histamine release. *J. Immunol.* **131**:1949–1953.
- Mori, A., Suko, M., Kaminuma, O., Inoue, S., Ohmura, Y., Nishizaki, Y., et al. 1996. IL-15 promotes cytokine production of human helper T cells. *J. Immunol.* **156**:2400–2405.
- Mori, A., Kaminuma, O., Suko, M., Inoue, S., Ohmura, T., Hoshino, A., et al. 1997. Two distinct pathways of interleukin-5 synthesis in allergen-specific human T-cell clones are suppressed by glucocorticoids. *Blood*. In press.