Der f 34, a Novel Major House Dust Mite Allergen Belonging to a Highly Conserved Rid/YigF/YER057c/UK114 Family of Imine Deaminases*

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The high prevalence of house dust mite (HDM) allergy is a growing health problem worldwide, and the characterization of clinically important HDM allergens is a prerequisite for the development of diagnostic and therapeutic strategies. Here, we report a novel HDM allergen that belongs structurally to the highly conserved Rid/YigF/YER057c/UK114 family (Rid family) with imine deaminase activity. Isolated HDM cDNA, named der f 34, encodes 128 amino acids homologous to Rid-like proteins. This new protein belongs to the Rid family and has seven conserved residues involved in enamine/imine deaminase activity. Indeed, we demonstrated that purified Der f 34 had imine deaminase activity that preferentially acted on leucine and methionine. Native Der f 34 showed a high IgE binding frequency as revealed by two-dimensional immunoblotting (62.5%) or ELISA (68%), which was comparable with those of a major HDM allergen Der f 2 (77.5 and 79%, respectively). We also found that Der f 34 showed cross-reactivity with another prominent indoor allergen source, Aspergillus fumigatus. This is the first report showing that the Rid family imine deaminase represents an additional important pan-allergen that is conserved across organisms.

Asthma is a serious global health problem that significantly reduces quality of life. In a cross-sectional World Health Organization survey of 178,215 individuals from 70 countries conducted in 2002–2003, it was estimated that 4.3% of adults (range, 0.2–21.0%) had been diagnosed with asthma by a medical doctor (1). Inhaled airborne allergens cause allergic inflammation via the cross-linking of allergen-specific IgEs bound on high affinity IgE receptor (FceRI) located on the surface of airway or lung mast cells, causing the secretion of inflammatory chemical mediators (e.g. histamine and leukotrienes) from activated mast cells. House dust mites (HDMs) and fungi are major sources of airborne allergens and trigger asthmatic attacks in allergic patients concomitant with air pollutants (2).

Allergen-specific immunotherapy (AIT) is the only curative treatment for allergic asthma that changes the natural course of an allergy (3). AIT induces allergen-specific tolerance by administering increasing doses of causative allergens subcutaneously or sublingually (4). The precise mechanisms underlying AIT remain to be fully elucidated, but the involvement of regulatory T cells and B cells or the induction of T cell anergy is suggested to be a key mechanism for an efficient AIT. AIT induces antigen-specific immunomodulatory cells or the anergic response of T cells specific to major allergens included in an allergen extract as an AIT vaccine. Therefore, the standardization of AIT vaccines using important major allergens and the characterization of major component allergens in an allergen source are important issues for the development of an effective AIT (5, 6).

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The nucleotide sequence(s) reported in this paper has been submitted to the DDBJ/GenBank™/EBI Data Bank with accession number(s) LC120618.

The sequence of Der f 34 has been submitted for registration in a database for the systematic allergen nomenclature approved by the World Health Organization and the International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee as the newly registered D. farinae allergen Der f 34.

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The abbreviations used are: HDM, house dust mite; AIT, allergen-specific immunotherapy; nDer f 34, native Der f 34; rDer f 34, recombinant Der f 34 without tag; rGST-Der f 34, rDer f 34 with GST tag; rTF-Der f 34, rDer f 34 as a trigger factor fusion protein; pAb, polyclonal antibody; ANOVA, analysis of variance; SNK, Student-Newman-Keuls.
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An HDM extract commonly used for AIT of HDM-allergic rhinitis and asthma is standardized by its concentrations of major allergens, Der f 1 and 2 or Der p 1 and 2. Only Der f 1/2 and Der p 1/2 are used for the standardization of a vaccine for AIT of HDM. On the other hand, many allergens have been identified and characterized from HDM, including Dermatophagoides farinae (American HDM) and Dermatophagoides pteronyssinus (European HDM), as Der f/Der p allergens or mite allergen gene (Mag) allergens, such as Der f 6 and Mag3 (7–14). Among the allergens, 28 from D. farinae have been formally registered in a database for systematic allergen nomenclature approved by the World Health Organization and the International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee. These allergens have been identified and characterized from mite body, mite feces, or a cDNA library of mite bodies. Der f 1 (cysteine protease), Der f 2 (MD2-like lipid-binding domain protein), and Der f 23 (peritrophin-like protein) are serodominant allergens in D. farinae (7, 15–17). Serine proteases from D. farinae, including Der f 3 (trypsin), Der f 6 (chymotrypsin), and Der f 9 (collagenase), act on mucosal epithelial cells to induce chemokine production that triggers the migration of inflammatory cells (13, 18–23). Tropomyosin as Der f 10 and gelsolin as Der f 16 are also important HDM allergens as pan-allergens (10, 14, 19).

We previously reported that subcutaneous AIT using an allergen fraction prepared from D. farinae feces extract (HM2 fraction) significantly reduced symptom-medication scores for bronchial symptoms without any severe adverse events, such as asthmatic attacks or anaphylaxis (24, 25). Interestingly, although the HM2 fraction has the highest allergenicity and tolerability, it does not contain major HDM allergens Der f 1 and Der f 2. This suggests that new HDM allergens, other than Der f 1 and Der f 2, are responsible for the immunotherapeutic effects of the HM2 fraction. These findings prompted us to characterize the component allergens in HM2 fraction, which should contain important uncharacterized allergens applicable to AIT.

In this study, we identified a novel allergen, Der f 34, belonging to the Rid/Yigf/YER075c/UK114 family (Rid family) with imine deaminase activity from HM2 fraction. The Rid family protein is broadly conserved from prokaryotes to mammals, and its characteristic sequence was referred to as (P/A)-(A/S/V/T/P/V)=-R-(S/A/C/V/F)-X-(L/I/V/M/F/Y)-X-(2)-(G/S/A/K/R)-X-(L/M/V/A)-X(5,8)-(L/I/V/M)-E-(M/I) (indicated by underlining in Fig. 1A), deposited as Prosite entry PS01094 on the Expasy server (Swiss Institute of Bioinformatics). The sequence also contained a domain conserved in the Yigf/YER075c/UK114 family, which was recently renamed the Rid family (26) and deposited in the NCBI conserved domain database (indicated in boldface type in Fig. 1A). These features of the deduced sequence show that Der f 34 is a new member of the Rid protein family.

Rid family proteins share catalytically critical arginine residues for their enamine/imine deaminase enzymatic activity and serve to hydrolyze the reactive enamine/imines generated by pyridoxal 5'-phosphate-dependent enzymes or FAD-dependent amine oxidases (27). The deduced amino acid sequence of Der f 34 contains seven amino acid residues (Tyr-19, Gly-33, Asn-59, Asn-91, Arg-105, Pro-114, and Glu-120; indicated in red in Fig. 1A) predicted to be involved in an active site structure or in a catalytic domain for enamine/imine metabolisms (27).

Der f 34 showed the highest identity with the Rid-like protein from marine γ-proteobacterium HTCC2143 (NCBI GenBank accession number EAW32813), with 57.0% identity and 81.2% similarity (28). The sequence of the Rid-like protein also contains the signature pattern for the UPF0076 family, a member of the Rid family (26). Der f 34 was also homologous to endoribonuclease from Halorhodospira halochloris (AHK77905), with 50.8% identity and 88.3% similarity; endoribonuclease (AEG00088) from Methylomonas methanica, with 50.4% identity and 80.5% similarity; and translation initiation inhibitor from Limnobacter sp. MED105 (EDM82235), with 46.9% identity and 81.2% similarity (Fig. 1B) (29, 30). Der f 34 is also homologous to enamine/imine deaminase from Candidatus acumulibacter (JDVG02000346) with 50.4% identity and 79.1% similarity, suggesting that Der f 34 could be an enamine/imine deaminase of D. farinae. Importantly, Der f 34 is also homologous to the UK114 protein from Homo sapiens (CAG33125), with 42.3% identity and 75.2% similarity, and to the Rid-like protein from Aspergillus ruber (EYE94158), with 39.1% identity and 73.4% similarity (Fig. 1B). These data suggest that proteins homologous to Der f 34 are broadly conserved in various species, including mammals, and that Der f 34 may act as a cross-reactive allergen in a variety of allergen sources.

**Results**

**Molecular Cloning and Characterization of der f 34 in D. farinae**—To identify uncharacterized allergens in the HM2 fraction, which has the highest allergenicity and tolerability in HDM extract, we immunoscreened a cDNA library of D. farinae with anti-HM2 fraction polyclonal antibody (pAb). Consequently, we cloned a cDNA named der f 34 from a cDNA library for D. farinae by immunoscreening with anti-HM2 fraction pAb. The cDNA for der f 34 consisted of 522 nucleotides containing a 387-bp open reading frame encoding a 128-amin acid polypeptide with a calculated isoelectric point of 6.25 and a molecular mass of 13,998 Da (Fig. 1A). The deduced amino acid sequence contained a characteristic pattern as an “uncharacterized protein family UPF0076 signature consensus pattern,” whose characteristic sequence was referred to as (P/A)-(A/S/V/T/P/V)=-R-(S/A/C/V/F)-X-(L/I/V/M/F/Y)-X-(2)-(G/S/A/K/R)-X-(L/M/V/A)-X(5,8)-(L/I/V/M)-E-(M/I) (indicated by underlining in Fig. 1A), deposited as Prosite entry PS01094 on the Expasy server (Swiss Institute of Bioinformatics). The sequence also contained a domain conserved in the Yigf/YER075c/UK114 family, which was recently renamed the Rid family (26) and deposited in the NCBI conserved domain database (indicated in boldface type in Fig. 1A). These features of the deduced sequence show that Der f 34 is a new member of the Rid protein family.

We made recombinant Der f 34 (rDer f 34) to prepare anti-rDer f 34 pAb available for immunoaffinity chromatography to purify native Der f 34 (nDer f 34). The rDer f 34 appeared at 16 kDa on SDS-PAGE, coinciding with a calculated molecular mass of 14 kDa (lane rDer f 34 in Fig. 2A). We immunized rabbits with the rDer f 34 for immunization.
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Aim: To identify a new allergen belonging to the Rid family of deaminases, which are known for their role in the degradation of proteins.

Methods: We performed a comprehensive bioinformatics analysis and conducted experiments to confirm the deaminase activity of the new allergen, Der f 34. The allergen was isolated from Der f 34-expressing bacteria, and its amino acid sequence was determined by mass spectrometry and protein sequencing. The enzymatic activity of Der f 34 was tested on various substrates, and its specificity and inhibition by known inhibitors were analyzed.

Results: Der f 34 possesses the typical structure of catalytic activity and the seven conserved amino acid residues for imine deaminase activity. It can hydrolyze various imine derivatives, including those with aromatic rings, in a manner similar to the known enzyme, RidAR105A. Der f 34 also shows homology with other imine deaminases, including those from bacteria, fungi, and mammals.

Conclusion: Der f 34 is a new allergen belonging to the Rid family of deaminases with imine deaminase activity. It could be a potential target for the development of new allergen-specific immunotherapies.

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residues (red characters in Fig. 1 A) are responsible for the imine deaminase enzymatic activity for Der f 34.

Potent IgE Binding Capacity of Der f 34 and Its Cross-reactivity with Fungal Allergens—We analyzed the importance of Der f 34 as an HDM allergen by allergenome analysis, which is an omics analysis for allergens capable of binding patients' IgE by two-dimensional immunoblotting (representative data is shown in Fig. 2 D) and ELISA using plasma from patients allergic to HDM. Our allergenome analysis revealed that a corresponding spot of nDer f 34 (indicated with an arrow in Fig. 2, C and D) showed positive binding with IgE in 25 of 40 patients (62.5%), which is comparable with that of Der f 2 (31 of 40; 77.5%) (data not shown). We further confirmed the IgE binding capacity of nDer f 34 by ELISA using plasma from 19 patients allergic to HDM. We defined IgE binding of nDer f 34 as positive if a patient's nDer f 34-specific IgE titer exceeded a threshold (indicated by the broken line in Fig. 4A) (mean + (3 x S.D.) of four healthy volunteers, 2.8 units/ml). 13 of 19 patients (68%) reacted positively with nDer f 34, whereas 15 of 19 (79%) reacted positively with a major allergen, Der f 2 (Fig. 4A) (data not shown). These data suggest that Der f 34 is a major allergen for HDM-allergic patients comparable with Der f 2.

Rid proteins are highly conserved from prokaryotes to mammals, and their amino acid sequences are highly homologous. Therefore, we analyzed whether Der f 34 shares its immunoglobulin-binding epitopes with those of other Rid family proteins. Importantly, Der f 34 showed significant homology with a Rid protein from a fungus (A. ruber), as indicated in Fig. 1 B. Fungi are also important sources of indoor allergens and trigger respiratory disorders, such as allergic asthma and aspergillosis (38). Therefore, we evaluated the cross-reactivity of Der f 34 and fungal allergens by an ELISA inhibition assay using allergenic fungal crude extracts and Der f 34-specific rabbit IgG (Fig. 4B). The crude fungal extract from A. fumigatus completely prevented the binding between rTF-Der f 34 and rabbit IgG specific to the rTF-Der f 34 in a dose-dependent manner, but the extract from Alternaria alternata did not inhibit the binding (Fig. 4B). These data strongly suggest that Der f 34 shares its immunoglobulin-binding epitopes with A. fumigatus allergens.
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Discussion

To achieve a more effective AIT for HDM allergy, it is important to characterize component allergens from the fraction of HDM that has the most potent allergenicity and tolerability by subcutaneous AIT. We characterized a novel allergen named Der f 34 as an imine deaminase belonging to the Rid family. This is the first report to show the IgE binding capacity of a Rid family protein in allergic patients. Der f 34 showed that imine deaminase activity involved in amino acid metabolisms cooperated with amino acid oxygenase or hydrogenase. Imine deaminase catalyzes the hydrolysis of imine to produce ammonia and ketone (27). Rid family proteins are highly conserved in various species, including prokaryotes and eukaryotes, and their sequences for the UPF0076 signature pattern and residues for enamine/imine deaminase activity are highly conserved (Fig. 1, A and B) (26). Due to sequence similarities among members of the Rid family, Der f 34 is a candidate for a novel pan-allergen in various allergen sources. We elucidated that Der f 34 shares its immunoglobulin-binding epitopes with those from Aspergillus species (Fig. 4B). This strongly suggests that Der f 34-specific IgE can bind with allergens that share their immunoglobulin-binding epitopes from other allergenic sources, because binding between epitopes and antigen-binding regions of immunoglobulins (Fab region) is crucial for antigen recognition of immunoglobulin, and this binding is independent of the immunoglobulin isotype (Fc region). Although we found its cross-reactivity only in Aspergillus species, it seems to cross-react with other allergens in the Rid family or with enamine/imine deaminases. Elucidation of the cross-reactivity of Der f 34 with other homologous allergens in various allergen sources may also be important for the precise diagnosis of HDM allergy.

High doses (100 and 300 μg/ml) of crude extract from A. fumigatus showed statistically significant inhibition of binding between Der f 34 and Der f 34-specific IgG, although an excess amount of a nonspecific negative control protein (rTF) failed to block the binding. This clearly indicates that the observed competition with rTF-Der f 34 as well as with A. fumigatus extracts is not a nonspecific binding. The present data also indicate that the nonspecific binding kinetics of rTF are distinct from those seen in rTF-Der f 34, D. farinae feces extract, and A. fumigatus extract (Fig. 4B). Our estimation from the inhibition assay suggests that the content of rDer f 34-cross-reactive allergens in A. fumigatus is only 0.001% in the crude extract. One possible reason for the low allergen content is that those cross-reactive allergens in A. fumigatus might be lost during the extraction procedures, because the Rid proteins are small molecules (<20 kDa), albeit the inhibition is statistically significant compared with that of A. alternata and rTF as a negative control. To gain further insight into the cross-reactivity, experiments using purified native A. fumigatus Rid proteins and Rid protein-deficient A. fumigatus extract (prepared by immunodepletion with anti-Der f 34 pAb or made from rid gene disruptants) are needed.

We characterized Der f 34 as a candidate for a major allergen available to develop a more efficient AIT vaccine. UK114 protein in the Rid family was reported to induce Th2-skewing responses in murine splenocyte and to induce high IL4 with low
**A New Allergen Belonging to the Rid Family of Deaminases**

IFN-γ production by costimulation with concanavalin A (39). We analyzed the potential for such an immunological skewing property of rDer f 34 produced by the COS-7 (monkey kidney fibroblast) expression system, which is a lipopolysaccharide-negative protein expression system, by culturing with murine splenocytes. However, we could not conclude that Der f 34 possesses the capacity to skew the Th2 response in mice (data not shown). It will be interesting to elucidate whether Der f 34 has the potency to skew the Th1/Th2 response in humans. Interestingly, UK114 protein was also identified in humans as a tumor-associated antigen (NCBI GenBank accession number P80601: 47.4% identical to and 75.2% similar to Der f 34), and human monoclonal antibody against the UK114 antigen showed complement-dependent cytotoxicity to tumor cell lines but not to normal cells, except for a small fraction of hepatocytes of fetal origin (40, 41). The UK114 protein also inhibits the growth of mammary carcinomas induced in Sprague-Dawley rats, and the authors of that report speculated that the UK114 antigen may be involved in antigen presentation and tumor immunosurveillance (42).

We tested cross-reactivity between rTF-Der f 34 and human recombinant GST-tagged UK114 but failed to find significant cross-reactivity as assessed by an ELISA inhibition assay and immunoblotting using anti-Der f 34 pAb (data not shown). One possible reason for this poor cross-reactivity is that UK114 was GST-tagged recombinant protein produced by the wheat germ cell-free protein expression system, whose conformation could be different from its natural counterparts. We could not clarify the cross-reactivity between native Der f 34 and native UK114 nor whether anti-Der f 34 antibody was involved in the progression or clinical condition of tumors or other human diseases. Further analysis is needed to clarify the cross-reactivity between Der f 34 and human UK114 protein. However, if native UK114 is cross-reactive antigen with Der f 34, it may possible that Der f 34-specific antibody might be involved in the mechanisms underlying the inverse association between an allergy and specific types of cancer (43). These reports implied that Der f 34 is capable of immunomodulation and may be involved in the therapeutic mechanisms of AIT.

Native Der f 34 had a high IgE binding capacity, comparable with that of Der f 2 (62.5 and 68% for Der f 34, 77.5 and 79% for Der f 2 by two-dimensional immunoblotting and ELISA, respectively; Fig. 4A) (data not shown), and Der f 34 cross-reacted with fungal allergens (*A. fumigatus*, Fig. 4B). These data strongly suggested that Der f 34 is an important cross-reactive allergen for HDM and other allergen sources and that the Rid family will be a new category for pan-allergens, such as profilins and nonspecific lipid transfer protein families (44). Allergen sources that digested amino acid in their metabolic pathways may contain enamine/imine deaminase in the Rid family homologous to Der f 34. Therefore, we consider that Der f 34 is also a clinically important major allergen for HDM-allergic rhinitis and asthma.

In this paper, we identified and characterized a novel mite imine deaminase, named *der f 34*, from HDM as a pan-allergen for the first time. Our results suggested that Rid family proteins are important as novel pan-allergens for the cross-reactivity among various allergen sources. It will be important to elucidate in the near future the contribution of Der f 34 to AIT using HDM extract.

**Experimental Procedures**

**Human Plasma—**HDM-allergic plasma samples were obtained from 19 patients selected on the basis of their clinical history and a positive CAP-RAST score for HDM (RAST score of 2 or higher). Non-allergic plasma from four healthy volunteers with negative RAST scores were used as negative controls. The study protocol conformed to the standards set by the Declaration of Helsinki. All experiments were approved by the institutional review board of Takanobashi Central Hospital and were described in detail to all participants before they provided their informed consent and were admitted into the study. The patients’ plasma samples for allergenome analysis (n = 40) and ELISA (n = 19) were collected separately for each experiment as a different data set.
A New Allergen Belonging to the Rid Family of Deaminases

Extraction of House Dust Mite Allergens—House dust mites (D. farinae) were cultured on Purina rodent chow (Orient Yeast, Tokyo, Japan) for 30 days, and the whole mite culture (1 kg) was suspended in a saturated sodium chloride solution (3 liters). The solution was centrifuged at 6,400 × g for 30 min, and supernatant was collected, dialyzed against water, and then lyophilized. The lyophilizate was used as mite feces extract. The mite feces extract was loaded onto a UF-20 ultrafiltration apparatus (Tosoh, Shin-Nanyo, Japan) to eliminate small molecular weight allergens. The resultant high molecular weight fraction was further fractioned by gel filtration chromatography using an Ultrogel AcA 54 column (Sigma-Aldrich) monitoring with an absorbance at 280 nm. A resultant high molecular weight fraction was further fractioned by gel filtration chromatography using an Ultrogel AcA 54 column (Sigma-Aldrich) monitoring with an absorbance at 280 nm. A resultant high molecular weight fraction was further fractioned by gel filtration chromatography using an Ultrogel AcA 54 column (Sigma-Aldrich) monitoring with an absorbance at 280 nm.

SDS-PAGE and Immunoblotting—The purified rDer f 34 and nDer f 34, the HM2 fraction, and the enriched nDer f 34 fraction after anion exchange chromatography were loaded onto 12.5% SDS-polyacrylamide gel under reduced conditions, and the proteins were visualized with silver staining following the manufacturer’s instructions (2D silver staining kit, Daiichi Kagaku Yakuhiin, Tokyo, Japan). Two-dimensional SDS-PAGE and allergenome analysis of D. farinae extract were performed using methods similar to those described previously (47). The two-dimensional immunoblotting analysis in the allergenomics study was carried out using plasma samples from 40 patients allergic to HDM. The HM2 fraction, the enriched nDer f 34 fraction, rDer f 34, and nDer f 34 were immunostained with 20,000-fold-diluted rabbit anti-rDer f 34 pAb, followed by 20,000-fold-diluted peroxidase-conjugated goat anti-rabbit IgG (Cell Signaling Technology, Danvers, MA), and visualized with ECL Plus Western blotting detection reagents (GE Healthcare Japan). The area of PVDF membrane corresponding to the protein weight marker was cut after protein transfer and then stained with Coomassie Brilliant Blue.

ELISA and ELISA Inhibition Assay—ELISA and ELISA inhibition were performed according to a method reported previously (48). Briefly, 25 ng/well nDer f 34 was coated onto a 96-well microtiter plate, which was then blocked with PBS containing 2% skim milk and 1% BSA. Serial dilution of purified human IgE (Athens Research Technology, Athens, GA) was coated on the plate as a calculator to estimate the positive IgE binding. Positive binding equal to the immobilized 10 ng of IgE was defined as 1 arbitrary unit. Then 50-fold-diluted plasma samples from 19 allergic patients or 4 healthy volunteers were added to the plate, followed by incubation with 1,000-fold-diluted biotin-conjugated anti-human IgE (e-chain-specific; BIOSOURCE International, Camarillo, CA). After incubation with 1,000-fold-diluted alkaline phosphate-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA), Attaphos substrate solution (Promega, Fitchburg, WI) was

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Purification of nDer f 34 from Crude Mite Extract—Two male rabbits were immunized with rDer f 34 with Freund’s complete adjuvant every 2 weeks eight times. Peripheral blood was drained, and serum containing pAb to rDer f 34 was collected after centrifugation with 10,000 × g for 20 min at 4 °C. Anti-rDer f 34 pAb was further purified using an rDer f 34-immobilized CNBr-activated Sepharose 4B column (GE Healthcare Japan). HM2 fraction (10 mg of protein) fractionated from mite feces extract was loaded onto a Resource Q anion exchange column (GE Healthcare Japan), and nDer f 34 was eluted with 0.1 M NaCl. The fraction containing 16-kDa molecules, which bound with anti-Der f 34 pAb by Western blotting analysis, and then the nDer f 34-enriched fraction were pooled and loaded onto an anti-Der f 34-immobilized protein G-Sepharose 4FF column (GE Healthcare Japan), and nDer f 34 was eluted with 0.1 M glycine HCl (pH 2.8). Animal experiments were carried out using protocols approved by the Animal Studies Committee of Hiroshima University.

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\[ \text{der f} 34 \] by substitution of seven amino acid residues responsible for enamine/imine deaminase activity (Tyr 19, G19, Asn-59, Asn-91, Arg-105, Pro-114, and Glu-120) by alanine.
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added to the plate, and fluorescence intensity was measured using a Wallac 1420 ARVOs multilabel counter (PerkinElmer Life Sciences). To analyze the cross-reactivity of Der f 34 with fungal allergens, rTF-Der f 34 was coated onto a microtiter plate and reacted with a 25,000-fold-diluted rabbit anti-HM2 fraction pAb preincubated with serial concentrations (300 μg/ml and 10-fold serial dilutions from 100 μg/ml) of fungal crude extract from A. fumigatus or A. alternata, crude extract from D. farinae feces, rTF-Der f 34, or rTF as a negative control. Both extract from A. fumigatus and A. alternata were purchased from ITEA (Tokyo, Japan). The percentage inhibition was calculated using the positive binding without inhibitor as 0% and saturated binding (average of 300, 100, 10, and 1 μg/ml) of rTF-Der f 34 as 100%.

Enzymatic Activity for Imine Deaminase of Der f 34—Imine deaminase activity was analyzed as reported elsewhere with slight modifications (26). Briefly, 300 nm rTF-Der f 34, mutant rTF-Der f 34, wild type RidA protein from S. enterica (NCBI GenBank™ accession number AF095578), inactive variant RidA<sup>R105A</sup> protein, or rTF as a negative control was mixed with 10 mM semicarbazide-HCl, 1 μg (2–5 units) of bovine liver catalase, 10 μg (30 millionths) of t-lysine acid oxidase from Crotalus adamanteus (Sigma-Aldrich), and substrate amino acid: 5 mM Leu, 6.25 mM Met, or 10 mM Phe in 50 mM potassium pyrophosphate (pH 8.7) (26, 37). A<sub>248 nm</sub> was monitored every 1 min for 15 min at 22 °C for the assay of enzymatic activity. The wild type RidA protein from S. enterica and inactive RidA<sup>R105A</sup> variant protein were prepared as described previously (27, 36). The amount of keto acid formation determined as A<sub>248</sub> was calculated as 0.6 × 10<sup>8</sup> M<sup>-1</sup> cm<sup>-1</sup> (49).

Statistical Analysis—Non-repeated ANOVA was applied to compare data among the groups, and, when the differences was significant (p < 0.01), post hoc analysis using a Student-Newman-Keuls (SNK) test was performed.

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Acknowledgments—The cDNA sequence for der f 34 (originally named magI33) has been registered in the DNA Data Bank Japan (DDBJ) with accession number LC120618 as Dermatophagoides farinae MAGI33 mRNA for imine deaminase. The sequence of Der f 34 has been submitted for registration in a database for the systematic allergen nomenclature approved by the World Health Organization and the International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee as the newly registered D. farinae allergen Der f 34.

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