

Induction of Allergic Reactions in Guinea Pigs with Purified House Dust Mite Allergens

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To establish a guinea pig model for house dust mite allergy with purified mite allergens, we studied the immune response to two major mite allergens, native Der f 1 (nDer f 1) and recombinant Der f 2 (rDer f 2) and crude mite extract in Hartley guinea pigs. Animals were immunized with either mite extract, nDer f 1 or rDer f 2, four times at 2- to 3-week intervals. Then the guinea pigs were examined as to the status of sensitization to the sensitizing antigen. Intradermal injection of mite antigens to mite extract-, nDer f 1-, and rDer f 2-sensitized animals induced both immediate and late-phase cutaneous reactions. Allergic airway disease was also provoked by the intranasal instillation of rDer f 2 or mite extract. Anti-nDer f 1 and rDer f 2 IgE as well as anti-mite extract IgE were produced in the sensitized guinea pigs and IgE titer for three mite antigens were comparable. We concluded that immunization of Hartley guinea pigs with nDer f 1 and rDer f 2 achieved sensitization to mite allergens, which was comparable to that obtained by the immunization with mite extract. A mite-allergic model suitable for immunological and pharmacological studies was established from rDer f 2-sensitized guinea pigs.

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Key Words: mite; recombinant Der f 2; native Der f 1; guinea pig; IgE.

INTRODUCTION

Hypersensitivity to house dust mites of the genus *Dermatophagoides* is a major component of allergic disease (1). Most of the new knowledge concerning the inflammatory process and medical treatment of house dust mite allergy, as well as the biochemical and immunological properties of mite allergens, has been obtained from animal studies using mice and guinea pigs that were immunized with crude body extract from house dust mites, *Dermatophagoides farinae* (Der f) and *Dermatophagoides pteronyssinus* (Der p) (2–6).

Since 1980, group 1 (Der f 1, Der p 1), group 2 (Der f 2, Der p 2), and other major allergens have been purified from extract of *Dermatophagoides* mites and used for immunological research (7, 8). Group 1 mite allergens have a molecular weight (MW) of 25 kDa and are glycoproteins with a cysteine-protease activity. Group 2 allergens are 14-kDa MW and have no sugar chain (9). No biological activities have been detected in group 2 allergens. Among the major house dust mite allergens, genes for Der f 1, 2, and 3 and Der p 1, 2, 3, 5, and 7 have been cloned (10, 11). In particular, the recombinant protein of Der f 2 has been shown to possess an IgE-binding activity comparable to the native Der f 2 and a method for the production of a large amount of rDer f 2 with a high level of purity has been established (12, 13). Since purified and recombinant mite allergens, such as native Der f 1 (nDer f 1) and recombinant Der f 2 (rDer f 2), have recently been marketed as reagents, these allergens can be substituted for crude mite extract in immunological research.

In a previous study, we showed that active sensitization of mice with nDer f 1 and rDer f 2, as well as crude mite extract, could induce antigen-specific IgE production (14). However, there have been no reports on the immune response to purified mite allergens in guinea pigs. In the present study, Hartley guinea pigs were immunized with nDer f 1, rDer f 2, or mite extract to induce IgE production, cutaneous reaction, and allergic airway reaction after antigen challenge. The sensitizing activities of nDer f 1 and rDer f 2 were compared with that of mite extract in guinea pigs and a mite-allergic model suitable for immunological and pharmacological studies was established from rDer f 2-sensitized animals.

MATERIALS AND METHODS

Antigen

Recombinant Der f 2, clone 1 (12), was supplied by the Nikka Whisky Distilling Co., Ltd. (Chiba, Japan).



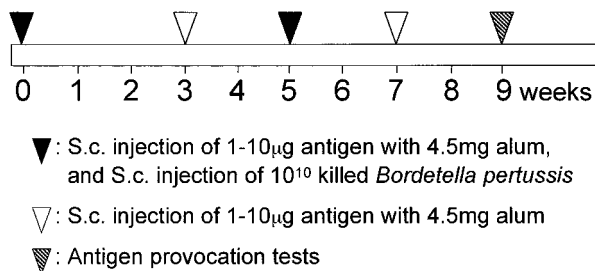


FIG. 1. Experimental schedule. Groups of eight guinea pigs were immunized with crude mite extract, nDer f 1, or rDer f 2 and challenged with a sensitizing antigen to elicit an allergic reaction.

Crude body extract from the house dust mite, *D. farinae* (mite extract), and native Der f 1 purified from mite culture extract were supplied by Torii & Co., Ltd. (Chiba, Japan). This mite extract contained approximately 0.3% (w/w) native Der f 1 and 0.3% (w/w) native Der f 2, as determined by enzyme-linked immunosorbent assay. Allergens were dissolved in phosphate-buffered saline (PBS) before use.

Animals

Specific pathogen-free male Hartley guinea pigs at 5 weeks of age were purchased from Japan SLC, Inc. (Shizuoka, Japan). The animals were housed in an air-conditioned room maintained at $24 \pm 2^\circ\text{C}$ and $55 \pm 5\%$ relative humidity with controlled illumination (lights on from 0600 to 1800). A commercial diet and water were available *ad libitum*. All animals were housed without any treatment for at least 1 week after arriving at our laboratory.

Immunization

Groups of eight 6-week-old guinea pigs were sensitized by subcutaneous injection of 4.5 mg of aluminum hydroxide (alum) and 1 μ g of nDer f 1, 10 μ g of rDer f 2, or 10 μ g of mite extract four times at intervals of 2–3 weeks (Fig. 1). In the first and third immunization, 10^{10} killed *Bordetella pertussis* (Wako Junyaku, Tokyo, Japan) were also injected subcutaneously as additional adjuvant to enhance IgE production (15). Several guinea pigs without immunization were used as negative controls.

The guinea pigs were examined for cutaneous reactions 2 weeks after the last immunization and 3–4 days later, they were subjected to airway provocation tests. The sensitized animals were intradermally and intranasally challenged with a sensitizing antigen alone, that is, mite extract, nDer f 1, or rDer f 2. Naive guinea pigs were divided into three groups and challenged with mite extract, nDer f 1, and rDer f 2, respectively.

Cutaneous Reactions

After measuring skin fold thickness of the shaved back of the guinea pigs by means of a thickness gauge as a "base thickness," 0.2 ml of 0.01–10 μ g/ml antigen solution was intradermally injected under light anesthesia with ethyl ether. After 20 min, skin fold thickness of each injected site was remeasured. An immediate cutaneous reaction was considered to be positive when the thickness after the injection of antigen solution exceeded two-fold of the base thickness value. The minimum concentration of antigen inducing a positive reaction was determined. The reaction declined within a few hours and another reaction with erythema and slight induration appeared thereafter in the sensitized animals. Twenty-four hours after the injection of the antigen solutions, the diameter of the erythema developed at each injected site by a late-phase allergic reaction was measured.

Airway Provocation Tests

The rise in specific airway resistance (sRaw) caused by immediate airway reaction after intranasal injection of either rDer f 2 or mite extract was determined by the double-flow plethysmograph method without anesthesia as described (16, 17). Briefly, thoracic movement and nasal flow of a guinea pig placed in a plethysmobox with two chambers for the head and body were determined with two pneumotachographs. Specific airway resistance was derived from the time delay (TD, in seconds) between thoracic movement and nasal flow under various alveolar pressures, that is, $\text{sRaw}(\text{cm H}_2\text{O} \cdot \text{s}) = (\text{RP}/2\pi) \cdot (760 \text{ mm Hg} - 47 \text{ mm Hg}) \cdot 1.36 \cdot \tan(2\pi\text{TD}/\text{RP})$, where RP is respiration time expressed in seconds.

When the respiration of the animal in the plethysmobox was stabilized, 0.05 ml of either 10 mg/ml rDer f 2 or 10 mg/ml mite extract solution was intranasally injected through a hole in the head chamber by means of a micropipette. In the rDer f 2-sensitized guinea pigs, sRaw was measured for 15–20 min from the intranasal challenge. Mite extract-sensitized animals were rechallenged with 0.05 ml of 30 mg/ml mite extract 15 min after the injection of 10 mg/ml solution, followed by an additional measurement of sRaw for 15 min. Due to the solubility of rDer f 2 in PBS, we did not use rDer f 2 solution with a concentration of more than 10 mg/ml. When sRaw exceeded $2.5 \text{ cm H}_2\text{O} \cdot \text{s}$, the immediate airway reaction was considered to be positive.

Twenty-four hours after provoking immediate reaction, the guinea pigs were anesthetized with ethyl ether and killed by bleeding from the inferior vein of the animals. Then tracheal cannulation was performed with a PE-250 polyethylene tube. Ten milliliters of PBS was injected into the lungs through the tube and the BALF (7–8 ml) was recovered. After counting the

total cell number in the BALF, cytospin preparations were made from 0.1-ml aliquots of the BALF and subjected to differential leukocyte counting after Dif-quick stain.

Due to difficulty in preparing large amounts of nDer f 1, inhalation challenge with nDer f 1 was not performed.

Eosinophil Viability-Enhancing Activity of Culture Supernatant of Spleen Cells

In order to determine eosinophil viability-enhancing activity (EVEA) of culture supernatant, dispersed spleen cells were prepared from sensitized and naive guinea pigs, which were not examined for cutaneous and airway reaction. Groups of five animals were newly immunized with mite extract, nDer f 1, or rDer f 2, as described above. Five naive guinea pigs without any treatment were used as controls. Spleen cells were prepared after obtaining heparinized whole blood from the inferior vein of each guinea pig for IgE and IgG determination. Plasma from five guinea pigs that received the same sensitizing treatment was pooled for each group (nDer f 1, rDer f 2, mite extract, and naive) and frozen until the antibody measurement. Dispersed spleen cells (5×10^6 cells) from the immunized animals were incubated in 1 ml of serum-free medium, AIM-V (Gibco, Grand Island, NY) in the presence or absence of $5 \mu\text{g/ml}$ of sensitizing antigen for 48 h. Cells from naive guinea pigs were cultured with or without mite extract, nDer f 1, or rDer f 2. Each culture supernatant was frozen at -70°C until the determination of EVEA.

Eosinophils were collected as described (18, 19). Briefly, 5-week-old Hartley guinea pigs were intraperitoneally injected with 1 ml of horse serum (Gibco) twice a week for 3 weeks. Twenty-four hours after the last injection, peritoneal exudate cells were collected and subjected to Ficoll-Paque (Pharmacia, Uppsala, Sweden) density gradient. Sedimentary cells were washed with AIM-V and incubated in culture dishes for 60 min. Nonadherent cells, in which the purity of eosinophils was more than 95%, were collected and used for the determination of EVEA.

Each spleen cell culture supernatant (SCCS) was fourfold diluted with AIM-V medium. The dilutes of AIM medium without antigen or with $5 \mu\text{g/ml}$ of mite extract, nDer f 1, or rDer f 2 were also prepared as the control. Eosinophils (5×10^4 cells) were cultured in 120 μl of the dilute in 96-well flat-bottom tissue culture plates for 72 h. Then, 15 μl of Alamar blue solution (Biosource, CA) was added to each well and eosinophils were subjected to additional culture for 4 h, followed by the measurement of fluorescent intensity. It has been shown that Alamar blue is reduced to become fluorescent in response to the metabolic activity of cells (20, 21). The fluorescent intensity of each well was used as an index of eosinophil viability in the well.

Measurement of Plasma Anti-mite Allergen IgE, IgG1, and IgG2 Levels

Pooled plasma obtained from naive and sensitized guinea pigs that were used for the preparation of spleen cells in the EVEA study was subjected to IgE and IgG measurement. Antibody levels for individual guinea pigs were not determined.

Immunoglobulin E levels specific for sensitizing antigen were determined by homogeneous 5-day passive cutaneous anaphylaxis (PCA) using guinea pigs at 12 weeks of age as recipients. One-hundred microliters of serial dilutions of plasma (1/10–1/3000) was intradermally injected into the shaved back of recipient animals under anesthetization with ethyl ether. After 5 days, 0.25 ml of 1% Evans blue solution containing 0.1 mg of the provoking antigen was intravenously injected into the recipients to provoke a PCA reaction. After 20–30 min, the maximum dilution at which a blue spot larger than 5 mm in diameter was induced was determined as the PCA titer, an index of antigen-specific IgE levels.

Anti-mite extract, -nDer f 1, and -rDer f 2 IgG subclasses were determined by ELISA following the recommendations of the manufacturers of the antibodies. Plasma was 1000-fold diluted with PBS containing 0.05% (w/v) Tween 20 and 5% bovine serum albumin fraction V before IgG determination. In the assays, we used mite extract, nDer f 1, or rDer f 2 as a capturing antigen and two detecting antibodies, peroxidase-conjugated goat anti-guinea pig IgG1 (GAGp/IgG1/PO, Nordic Immunological Laboratories, Tilburg, the Netherlands) and goat anti-guinea pig IgG2 (GAGp/IgG2/PO, Nordic Immunological Laboratories). 2,2'-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and H_2O_2 were used as substrates for color development. Diluted plasmas were reacted in the well (i) with capturing antigen or (ii) without antigen. Delta absorption at 405 nm (ABS 405 nm), that is, the difference between ABS 405 nm of (i) and (ii), was calculated, and IgG levels were expressed by $\Delta\text{ABS 405 nm}$.

Statistical Analysis

All of data obtained are expressed as means with SEM. Student's paired *t* test was used to compare differences in the measurements of airway reactions and leukocyte analyses between pairs of groups. A *P* value lower than 0.05 was considered significant.

RESULTS

Cutaneous Reaction

After four immunizations, groups of eight guinea pigs were examined for cutaneous reaction provoked by sensitizing mite antigens. The provocative concentra-

TABLE 1
Provocative Concentration of Mite Antigens
for Immediate Cutaneous Reactions

Antigen ^a	Antigen concentration (μg/ml)				
	0.01	0.1	1	10	>10 ^b
Mite extract		■	■■■■	■■	■
nDer f 1	■■	■■	■■	■	■
rDer f 2		■■	■■	■■■	■

Note. Individual guinea pigs are represented with “■”.
^a Used for both immunization and provocation of the reaction.
^b Did not react to antigen under 10 μg/ml.

tion of antigens for immediate reactions are shown in Table 1. Seven of eight guinea pigs immunized with mite extract reacted to 10 μg/ml of mite extract solution and this was the case in both nDer f 1- and rDer f 2-sensitized animals. The provocative concentration of nDer f 1 was slightly lower than that of the mite extract and rDer f 2. There was no difference between the mite extract- and rDer f 2-induced reactions. Naive guinea pigs did not react to mite antigens (data not shown).

After recovery from the immediate reaction, cutaneous erythema caused by a late-phase reaction appeared in all sensitized animals irrespective of the kind of mite antigen. Since the reaction was maximized around 24 h after the injection of antigen solution (data not shown), we measured the diameter of the erythema at this time point (Fig. 2). In the low concentration of provoking antigen (0.01–0.1 μg/ml), nDer f 1-induced a late-phase reaction that was more severe than the mite extract- and rDer f 2-induced reactions, as well as an immediate reaction.

Allergic Airway Reaction

The guinea pigs were challenged with either rDer f 2 or mite extract to induce immediate airway reaction 3–4 days after provoking a cutaneous reaction. After an intranasal instillation of rDer f 2 and mite extract, four and five of eight sensitized animals showed positive reactions with a rise in sRaw, respectively. No allergic symptoms were observed in naive animals challenged with mite extract or rDer f 2. Changes in the sRaw after intranasal antigen challenge in sensitized and naive animals are shown in Fig. 3. The increase in sRaw after intranasal challenge with 10 mg/ml of rDer f 2 was higher than that with 10 mg/ml of mite extract. Mite extract-sensitized animals were further challenged with 30 mg/ml of antigen and sRaw increased in a dose-dependent manner.

Twenty-four hours after intranasal challenge with either mite extract or rDer f 2, BALF was collected from each animal. As shown in Fig. 4, marked numbers of eosinophils were found in the BALF of sensitized guinea pigs showing positive immediate airway reaction, irrespective of the kind of mite antigen. This was not the case in naive animals challenged with mite antigen and sensitized animals challenged with PBS (data not shown). Increases in leukocytes were also found in the BALF taken from sensitized guinea pigs that did not show positive immediate reaction and their leukocyte counts were intermediate between naive and reaction-positive animals. Leukocyte influx provoked with mite extract and rDer f 2 was comparable.

EVEA of Culture SCCS

Under all conditions examined, EVEAs ordered as follows: SCCS from sensitized animals > SCCS from naive > medium alone (Fig. 5). EVEA values of sensitized guinea pigs were significantly increased by stim-

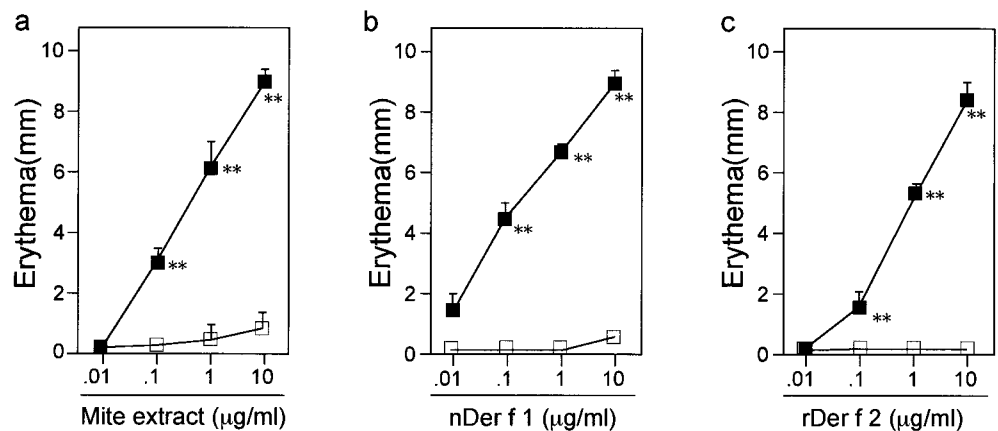


FIG. 2. Late-phase cutaneous reaction provoked 24 h after an intradermal injection of sensitizing antigen in sensitized (■, *n* = 8 in (a) to (c)) and naive (□, *n* = 4) guinea pigs. (a) Mite extract-induced reaction in animals immunized with mite extract, (b) nDer f 1-induced reaction in nDer f 1-sensitized animals, and (c) rDer f 2-induced reaction in rDer f 2-sensitized animals. ***P* < 0.01 compared with naive.

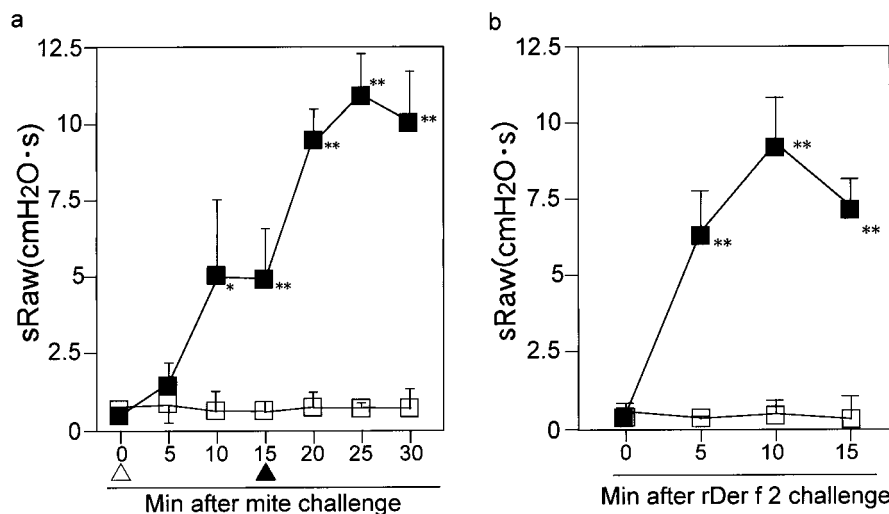


FIG. 3. Immediate airway reaction provoked with mite antigen in sensitized (■, $n = 5$ in (a) and $n = 4$ in (b)) and naive (□, $n = 4$) guinea pigs. Data from sensitized animals that did not show positive reactions are omitted. (a) Mite extract-induced reaction in animals immunized with mite extract. Animals were sequentially challenged with 10 mg/ml (△) and 30 mg/ml (▲) of mite extract solution; (b) rDer f 2-induced reaction in rDer f 2-sensitized animals. Animals were intranasally challenged with 10 mg/ml of rDer f 2 solution. ** $P < 0.01$ and * $P < 0.05$ compared with naive.

ulation of the spleen cells by a sensitizing antigen, that is, mite extract, nDer f 1, or rDer f 2. The type of mite antigen had little effect on the EVEAs in the sensitized animals. Concerning naive guinea pigs, nDer f 1 stimulation significantly raised the EVEA of SCCS and rDer f 2 stimulation slightly increased it. This tendency was also seen in the EVEAs of medium with or without mite antigens. Under stimulation with mite extract and rDer f 2, EVEAs of sensitized guinea pigs were significantly higher than that of naive ones. However, this was not the case under stimulation with

nDer f 1, because of the increase in the EVEA value of naive guinea pigs.

Plasma Anti-mite IgE, IgG1, and IgG2 Levels

Pooled plasma of guinea pigs used for the EVEA study was examined for antigen-specific IgE, IgG1, and IgG2 levels. Regarding IgE, the 5-day homogeneous PCA titer for each mite allergen was 300 and IgE production to the three mite antigens was comparable in Hartley guinea pigs. No IgE production to Der f 1 and Der f 2 was detected in mite extract-sensitized guinea pigs, though the mite extract contained a small amount of nDer f 1 and native Der f 2.

Anti-mite antigen IgG1 and IgG2 levels in the pooled plasma were determined by ELISA (Fig. 6). Mite extract-, nDer f 1-, and rDer f 2-sensitized guinea pigs showed production of both IgG1 and IgG2 specific for sensitizing antigens, which was not observed in naive animals. In all antigens, Δ ABS values for IgG1 were higher than that for IgG2. In addition, an order (rDer f 2 > mite extract > nDer f 1) appeared in both Δ ABS values for IgG1 and that for IgG2. However, it was unclear whether these differences in Δ ABS reflected the differences in the absolute concentration between IgG1 and IgG2 or among anti-rDer f 2, anti-mite extract, and anti-nDer f 1 IgG antibodies.

We also determined antigen-specific antibody levels in pooled plasma of the sensitized guinea pigs which were used for antigen provocation tests. Although the animals were challenged with sensitizing antigen 1–4 days before bleeding, results were comparable to those of animals used for the EVEA study without antigen challenge (data not shown).

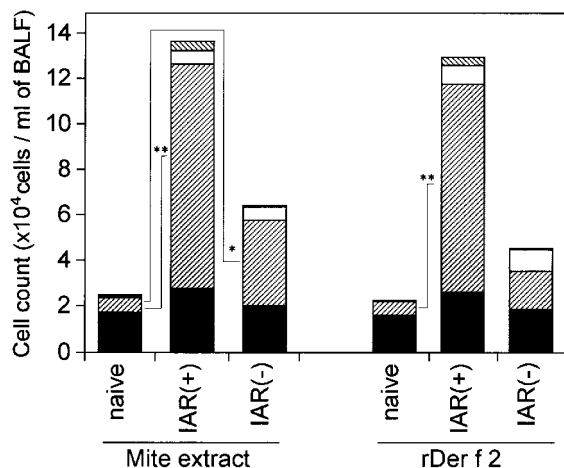


FIG. 4. Leukocyte count in the BALF 24 h after intranasal instillation of a mite antigen in naive, immediate airway reaction-positive sensitized guinea pigs (IAR(+)) and negative ones (IAR(-)). Macrophage (■), eosinophil (▨), neutrophil (□), and lymphocyte (▤). Sensitized animals were challenged with sensitizing antigen, either mite extract or rDer f 2. ** $P < 0.01$ and * $P < 0.05$.

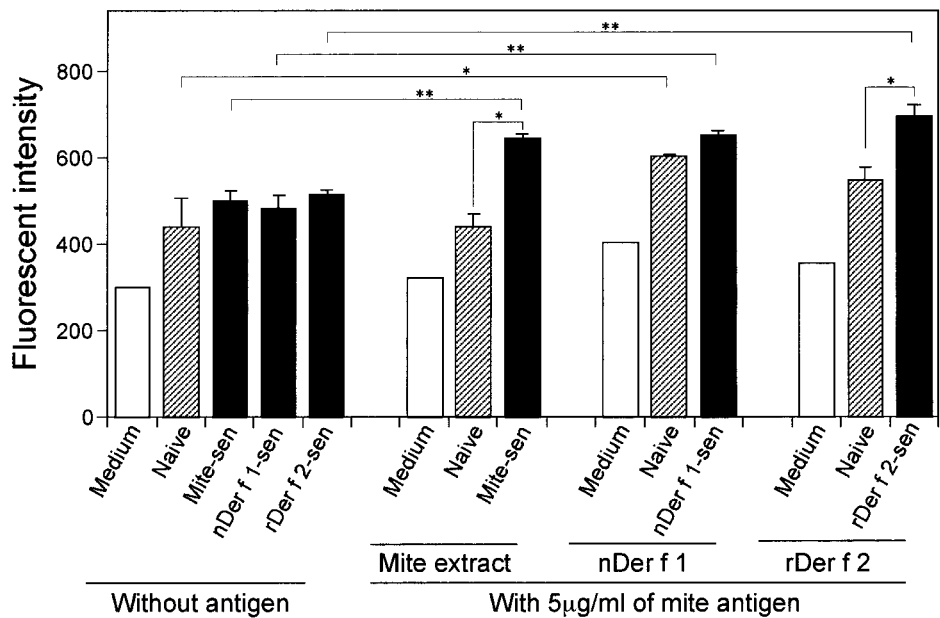


FIG. 5. Eosinophil viability-enhancing activity of culture supernatant of spleen cells and medium. sen, sensitized guinea pigs. Each bar for sensitized and naive groups represents the mean of EVEA of five culture supernatants. Spleen cells were cultured with or without 5 µg/ml of a mite antigen for 48 h. Antigen-free and -containing medium were also incubated for 48 h at 37°C. Eosinophils were cultured for 72 h in the fourfold dilute of medium or culture supernatant. Freshly isolated eosinophils without 72-h culture showed a fluorescent intensity value around 1000 (data not shown). ***P* < 0.01 and **P* < 0.05.

DISCUSSION

In industrialized countries, considerable numbers of people exhibit allergic symptoms caused by type I allergies and recently the number of allergic patients needing medical care has increased considerably (22, 23). Any approach to the experimental study of allergic diseases to improve medical treatment requires a suitable animal model, such as mouse, rat, or guinea pig. Concerning house dust mite allergy, two models showing severe eosinophil infiltration of the airway have recently been established using mice and guinea pigs with crude mite extract as the sensitizing allergen (5, 6). However, the contents of major and minor allergens in various mite extracts differ from supplier to supplier. In addition, mite extract contains antigenic proteins and substances, which can cause nonallergic inflammation by activating complement or platelet-activating factor (24–26), of various concentrations. Sensitizing experiments must be performed using the same mite extract in order to obtain experimental reproducibility. However, purification of major mite allergens and production of recombinant mite allergens have somewhat improved the situation. Purified mite allergens are now being substituted for mite extract in diagnosis, immunotherapy of house dust mite allergy and basic research into allergies and immunology. In the present study, the usefulness of rDer f 2 and nDer f 1 was clearly shown by sensitization experiments in guinea pigs.

After immunization with purified mite antigens, nDer f 1 and rDer f 2, Hartley guinea pigs were sensitized to show skin reactions upon antigen provocation. The severity of the immediate and late-phase cutaneous reactions provoked by purified antigens were equal or higher compared to mite extract-induced reactions. Preliminary histochemical study revealed severe accu-

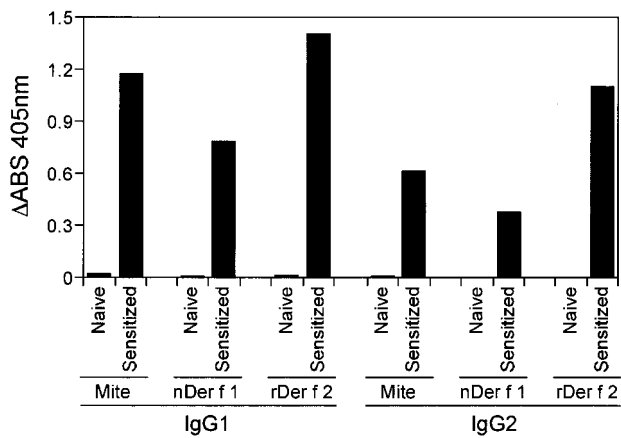


FIG. 6. Anti-mite antigen IgG1 and IgG2 levels in sensitized and naive guinea pigs. Pooled plasma obtained from mite-, nDer f 1-, and rDer f 2-sensitized animals that were used in the EVEA study was subjected to the measurement of antibody titer specific for mite extract, nDer f 1, and rDer f 2, respectively. Pooled plasma of five naive animals was used as a negative control for each antibody titer. Antibody levels for individual guinea pigs were not determined.

mulation of eosinophils and neutrophils at the site of the cutaneous reaction irrespective of the kind of mite antigen (data not shown), which is known as one of the most common pathological features observed in atopic dermatitis in humans (27–29).

In a previous study, we showed that A/J and C3H/He mice that were immunized with rDer f 2 showed immediate bronchoconstriction after inhalation of rDer f 2 (14). However, due to the small size and weak airway reactivity in mice, a direct connection between a respirator and the mouse trachea by tracheal cannulation was necessary to accurately measure bronchoconstrictive response. Thus sensitized mice could not survive after the measurement of bronchoconstriction and antigen provocation tests could not be repeated in rDer f 2-sensitized mice. In addition, it has been reported that the mouse airway is resistant to several mediators, such as histamine, leukotrienes C4 or D4 and substance P (30, 31). These properties of mice make it difficult to use murine asthmatic models for the pharmacological studies of antiallergic drugs which are expected to antagonize the above mediators. In contrast, reactivity and sensitivity of the guinea pig airway to physical and chemical stimuli are very high and similar to those of human allergic patients (24, 32). Hyper-responsiveness of the airway makes guinea pigs the most used animal in the evaluation of antiallergic drugs (33–35). After immunization with rDer f 2, half of the Hartley guinea pigs were sensitized to rDer f 2 to show immediate airway reaction after intranasal injection of rDer f 2 and rises in sRaw caused by the reaction were comparable to those of guinea pigs which were sensitized and challenged with mite extract or ovalbumin (OA) (36, 37). In addition, intense eosinophil influx into the airway was observed 24 h after intranasal challenge. The rDer f 2-sensitized guinea pigs shared most of the allergic symptoms of severe mite-allergic patients. As the airway and the cutaneous reaction could be provoked recurrently at 1-week intervals in rDer f 2-sensitized animals without a decrease in the severity of the reaction for at least 5 weeks (data not shown), they should also prove useful in pharmacological studies, as well as mite extract and OA-sensitized animals.

It has been shown that eosinophil accumulation, that seems to be mediated by a number of cytokines, is associated with the pathology and symptomatology of allergic diseases in clinical and experimental studies (39–41). Sputum or culture supernatant of peripheral blood leukocytes, that was obtained from patients with bronchial asthma or atopic dermatitis, had an elevated EVEA compared to those from healthy people (42, 43). We also found that EVEAs of SCCS of mite extract-, nDer f 1-, and rDer f 2-sensitized guinea pigs were higher than that of naive animals. Although we have yet to determine what type of cytokine in the SCCS related to the EVEA, granulocyte–macrophage colony-

stimulating factor and interleukin-5 have been identified as eosinophil-activating cytokines in human sputum (44). These cytokines could be produced by activated T cells that were reactive to the sensitizing antigens. Although lack of antibodies directed to guinea pig cytokines hinders the analysis of SCCS, several guinea pig cytokines have had their genes cloned (45, 46). Spleen cells of guinea pigs can be examined for their cytokine gene expression at RNA level.

In addition to successful induction of the allergic reactions, antigen-specific IgE, IgG1, and IgG2 production was elicited in Hartley guinea pigs after immunization with purified mite antigens, nDer f 1 and rDer f 2, and crude mite extract. Concerning IgE, the PCA titer became 300 in both nDer f 1- and rDer f 2-sensitized animals, which was comparable to IgE production elicited by immunization with mite extract. High antigenicity of nDer f 1 and rDer f 2 was clearly shown by the inducibility of the high IgE production, as well as that of the severe allergic reactions. In guinea pigs, both IgE and IgG1 antibodies can mediate immediate and late-phase allergic reactions, while the participation of IgG2 in the reaction is still unclear (47–51). Passive sensitization studies showed that IgE- and IgG1-mediated allergic reactions had different properties ranging from sensitivities to drug pretreatment and involvement of the kind of chemical mediators, which suggested the variety of the IgE and IgG1 involvement in individual allergic reactions (52–55). In order to clear the relationship between allergic reactions and antibody levels in our guinea pig models, measurement of IgE, IgG1, and IgG2 levels in individual animals, as well as allergic skin and airway reactions, will be needed.

Native Der f 1-induced cutaneous reactions were slightly more severe than mite extract- and rDer f 2-induced ones, though plasma PCA titer of mite extract-, nDer f 1-, and rDer f 2-sensitized animals was comparable. In addition, EVEAs of nDer f 1-containing medium and culture supernatant of nDer f 1-treated naive spleen cells were apparently higher than their counterparts of mite extract and rDer f 2. Group 1 mite allergens have been reported to increase airway mucosal permeability by their proteolytic activities (56). More recently, it has been shown that Der p 1, homolog of Der f 1 in Der p mite, could bias the immune response toward Th2 dominant, which is essential for the pathogenesis of type 1 allergy, by cleaving membrane CD23 molecules on B cells and α subunit of the IL-2 receptor (IL-2R or CD25) on activated T cells (57, 58). Cystein-protease activity of nDer f 1 may contribute to the above phenomena observed in the present study. We would like to undertake further experiments that characterize nDer f 1 in species other than guinea pigs, in addition to the present study.

We performed subcutaneous injection and intranasal instillation of the antigen solution to immunize animals and to provoke allergic airway disease. As a result, half of the sensitized animals did not show immediate airway reaction after intranasal antigen challenge. Since experimental animals should not be wasted, efforts are needed to increase the number of animals that exhibit a positive reaction. In addition, eosinophil accumulation in the airway of mite-sensitized guinea pigs was observed 24 h after intranasal challenge; however, we could not detect the increase in sRaw 6–24 h after the challenge (data not shown), which has been frequently observed in OA-sensitized guinea pigs after inhalation of OA (59, 60). It has been reported that repeated inhalation challenge with an antigen, as a sensitizing method, induced airway hyperresponsiveness efficiently and could sensitize guinea pigs to show late-phase bronchoconstriction (34, 61, 62). Antigen inhalation deserves to be tested, though this method requires a larger amount of mite antigens which are currently expensive.

In conclusion, it was clearly shown that nDer f 1 and rDer f 2 had high antigenicity that was comparable to mite extract in guinea pigs. At the same time, experimental allergic models were established in guinea pigs using rDer f 2 and nDer f 1 as the sensitizing antigens. These models and purified mite allergens will contribute to the development of research into allergies and immunology.

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