

SPECIFIC IgA AND IgG ANTIBODIES TO HOUSE DUST MITE
DERMATOPHAGOIDES FARINAE IN NASAL SECRETIONSShui Fang Xiao, Minoru Okuda, Masaki Ohnishi and Kimihiro Okubo
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As far as we known, IgA and IgG antibodies to purified house dust mite allergens *Der f I* and *Der f II* in nasal secretions have never been documented. Therefore, we determined specific IgA, SIgA and IgG antibodies (abs) to crude extract of *D. farinae* and its purified allergens *Der f I* and *Der f II* in nasal secretions collected by aspiration from 34 normal subjects, 25 untreated nasal allergic patients and 28 treated nasal allergic patients on parenteral immunotherapy by means of an avidin-biotin ELISA. The following results were obtained. (1) The specific IgA, SIgA and IgG abs to each of the three kinds of allergens correlated with each other. The groups of patients with nasal allergy (both treated and untreated) showed higher levels of specific IgA, SIgA and IgG abs to the allergens than the normal group. (2) In the group of treated patients, the levels of specific abs were not correlated with the clinical improvement of symptoms or the degree of response to nasal challenge. (3) The treated patients failed to show significantly higher levels of abs in nasal secretions than the untreated patients. (4) The specific IgA and SIgA abs in nasal secretions seemed to be predominantly produced locally, and IgG abs might be transudated from the circulation.

Key words: mite allergen *Dermatophagoides farinae*, *Der f I* and *Der f II* — nasal allergy — nasal secretions — parenteral immunotherapy — secretory IgA and IgG antibodies

INTRODUCTION

Exocrine secretions on the surface of mucosa are one inseparable part of mucosal immunity, and different classes of immunoglobulins, especially secretory IgA (SIgA), are the most important components¹⁾²⁾. Nasal mucosa can respond to influenza viruses and bacterial infection resulting in the production of protective IgA and IgG antibodies^{3)–5)}. The nasal mucosa also responds to various inhaled airborne allergens. In pollinosis, patients allergic to various kinds of pollens show higher levels of pollen-specific IgA and IgG abs in

their nasal secretions^{6)–8)}. On the other hand, the effects of parenteral immunotherapy with relevant pollen extracts are still controversial; it enhanced local specific IgA ab and IgG ab production⁶⁾⁸⁾ or had little influence on local increases in ab⁹⁾ or had no effect on IgA ab but enhanced IgG ab production in nasal secretions¹⁰⁾. Local administration of timothy grass pollen induced a significant increase in specific IgA, IgG and IgE abs in nasal secretions¹¹⁾.

Specific SIgA and IgG ab levels in nasal secretions have also been examined in perennial allergic rhinitis. House dust mite *Dermatophagoides farinae* (*Der f*) and *Dermatophagoides pteronyssinus* (*Der p*) are the main species of causative allergens in house dust¹²⁾¹³⁾. Patients allergic to house dust mite extract of *D. farinae* showed higher levels of IgA and IgG abs in nasal secretions than pollinosis patients¹⁴⁾ and normal controls¹⁵⁾¹⁶⁾. Parenteral immunotherapy produced no effect on the level of specific IgA ab¹⁴⁾ and IgG ab¹⁵⁾ in nasal secretions. These studies have been mostly carried out by classical methods for the determination of specific abs using non-purified crude allergens and nasal secretions collected by nasal washing.

Received: February 17, 1994

Accepted: March 22, 1994

Abbreviation: Abs antibodies; BSA bovine serum albumin; EIA enzyme-linked immunoassay; ELISA enzyme-linked immunosorbent assay; *D. farinae* *Dermatophagoides farinae*; *Der f I* *Dermatophagoides farinae* group I allergen; *Der f II* *Dermatophagoides farinae* group II allergen; HPLC high performance liquid chromatography; Ig immunoglobulin; PBS phosphate buffered saline; PBS-T PBS-Tween 20; RAST radioallergosorbent assay; SIgA secretory IgA

The purified *Dermatophagoides farinae* group I allergens (*Der f* I) and *Dermatophagoides farinae* group II allergen (*Der f* II) are the most important components of *Der f* mite allergen¹⁷⁾¹⁸⁾ and recently reported research into antibody levels to the two allergens has focused only on serum IgG and IgG subclasses¹⁹⁾²⁰⁾. As mentioned above, the levels of specific SIgA and IgG abs in nasal secretions have already been studied in connection with pollinosis and crude house dust mite extract of *D. farinae*, but little work has been done on purified mite allergens since they have only recently become available. Thus, the purpose of the present study was to clarify the relative concentrations of *Der f* I and *Der f* II specific SIgA and IgG abs in nasal secretions collected by aspiration and determined by an up-to-date sensitive avidin-biotin ELISA and to compare normal groups with allergy groups, and untreated allergy groups with treated (immunotherapy) allergy groups.

To determine whether specific IgA and IgG abs in nasal secretions might be part of a generalized secretory response to inhaled allergen, we examined the saliva as well.

MATERIALS AND METHODS

Subjects and collection of specimens

Fifty-three patients with nasal allergy to house dust mite were included in the present experiments. All of them were positive in the tests for mite nasal allergy, which included clinical history, rhinoscopic examination, nasal smear for eosinophilia, intradermal test, nasal provocation test and determination of specific mite IgE antibodies by radioallergosorbent test (RAST). Quite a few patients with mite allergy also showed positive results in the tests for Japanese cedar pollinosis. Twenty-five of them, aged 8 to 70 years with a mean age 27 years, who had never been treated before with any kind of immunotherapy, were defined as the untreated group. The remaining twenty-eight patients, aged 10 to 57 years with a mean age 27 years, who are currently undergoing parenteral immunotherapy in our hospital, were categorized as the treated group. Thirty-five normal volunteers, aged 10 to 57 years with a mean age 33 years, who were free from any history of

nasal disease were regarded as the normal control group. Samples were obtained from the 3 groups during the same period between June and September (out of the Japanese cedar pollen season) 1993. Nasal secretions were carefully collected from the nasal cavity by suction so as to cause minimal stimulation or injury to the nasal epithelium. To determine the precise amount of the nasal secretion collected, the test tubes containing the nasal secretions were weighed with a fine scale (sensitivity 0.01 mg) before and after collection. The nasal secretions were immediately diluted 100 times with 0.1 M phosphate buffered saline (PBS) pH 7.2, mixed with a vortex for 10 minutes and then centrifuged at $3,000 \times g$ for 30 min. The supernatant thus obtained was collected and stored at -40°C until use.

Whole unstimulated mixed saliva was obtained by aspiration and was processed in the same way as the nasal secretions. To determine specific IgA, IgG and IgE abs in serum, blood samples were also collected at the same time as the nasal secretions were taken in 16 untreated allergic patients.

Immunotherapy

The patients were given subcutaneous injections of an aqueous extract of house dust (the house dust allergen extract of 1:100 dilution contained 22 ng/ml *Der f* I and 1.2 ng/ml *Der f* II, respectively) as described previously¹⁴⁾. Briefly, the initial concentration was a 10-time dilution of the threshold concentration determined in intracutaneous tests i.e. mostly $1:10^4$ to $1:10^6$ dilutions of the stock solution. The dose was gradually increased and reached a maintenance dose of $1:10^2$, 0.5 ml (in a few cases $1:10$, 0.5 ml) after about half a year. The intervals of injections were once or twice a week at the beginning and finally monthly or once every two months. The maintenance dose was continued for a long period: the average period was three years and five months (7 months to 10 years), and the average dose was about 16.86 ml (0.03–60.21 ml) of house dust extract (1:100), which consisted of about 387 ng (0.7 to 1324.6 ng) of *Der f* I and 23 ng (0.04 to 72.3 ng) of *Der f* II.

Clinical improvement was evaluated by changes in the severity of symptoms and the sensitivity in nasal challenges to house dust extract before and

after immunotherapy. The classification of severity of symptoms (0, +, ++ and +++ respectively) and testing methods are described elsewhere²¹⁾.

Assays of protein and specific abs in nasal secretions

Total protein: Total protein was determined by the bichinchonic acid method (BCA Protein Assay Kit, Pierce, USA).

A 96-well immunomicroplate (NUNC, Denmark, high binding capacity) was selected for enzyme-linked immunosorbent assay (ELISA). Carbonate-bicarbonate buffer, (0.1 M, pH 9.6) was used as coating buffer, 0.05% Tween 20–0.1 M PBS (PBS-T) as washing buffer and 0.5% Tween 20–0.1 M PBS–0.5% bovine serum albumin (BSA, Wako, Japan) (PBS-T–BSA) as diluent, and the volume of samples or reagents used during incubation was 100 μ l/well throughout all the ELISA assays.

Albumin: Human serum albumin was measured by an indirect non-competitive ELISA by the method of Engvall and Permamn²¹⁾. Rabbit anti-human albumin (Dako, Denmark) as the first coating ab, 2% rabbit serum (Sigma) as the blocking agent, standard human albumin (Sigma), peroxidase-conjugated rabbit antihuman albumin (Dako, Denmark) and *o*-phenylenediamine dihydrochloride substrate (Sigma) were consecutively incubated, followed by the addition of 2 M H₂SO₄ as a stopping solution. Optical density was measured at 490 nm. The assay range was between 1 and 500 ng/ml.

Total IgA: Total IgA was assayed by indirect, non-competitive ELISA as a modified method combining ELISA²²⁾ with an avidin-biotin technique²³⁾. Goat antihuman IgA (Dako), diluted to 24 μ g/ml was plated at 4°C overnight. The uncoated sites were blocked with 1% BSA for 1 hr at 37°C. Standard secretory IgA (Organon, Teknika Corp., West Chester, PA) or diluted samples (1 : 5 $\times$ 10⁴ dilution from original specimen), goat antihuman IgA biotin conjugate (Sigma) (diluted to 1 : 5 $\times$ 10⁴), streptavidin- β -D-galactopyranoside substrate (Sigma) were consecutively incubated at 37°C for 2 hr, followed by the addition of 1 M Na₂CO₃ as a stopping agent. Optical density was measured at 415 nm. Following each step, the plates were washed 5 times. The substrate was dissolved in 20 nM PB buffer, pH 7.2 at a concentration of 5 nM, containing 0.1 M NaCl and 1 nM MgCl₂. The range

of sensitivity was 0.5 to 500 ng/ml.

Secretory IgA: Instead of IgA ab, rabbit anti-human secretory components (Dako), diluted to a concentration of 34 μ g/ml were coated. Subsequent steps for this assay were identical to the total IgA assay outlined above. The assay range was 0.5 to 500 ng/ml.

IgG: The entire procedure for this assay was identical to that for the total IgA assay. Goat antihuman IgG (Sigma, Fab specific) 10 μ g/ml, standard IgG (Sigma) and goat antihuman IgG biotin conjugate (γ -chain specific) at a dilution of 1 : 3 $\times$ 10⁴ were used. The assay range was 1 to 500 ng/ml.

Allergens: Crude extract of *Dermatophagoides farinae* (*D. farinae*), and purified allergens *Der f* I and *Der f* II were obtained from Research Laboratories, Torii & Co., LTD, Ichikawa, Japan. The methods of purification are described elsewhere¹⁷⁾²⁴⁾. The purity of these two allergens was about 95%. The amounts of *Der f* I and *Der f* II in house dust extract (immunogen for immunotherapy and in vivo diagnosis such as intradermal skin test and nasal provocation test) and in the crude extract of *D. farinae* were determined by an ELISA assay similar to the total IgA assay. Briefly, rabbit anti-*Der f* I and *Der f* II sera were precipitated with 50% saturated ammonium sulfate and dialyzed. The IgG fraction was isolated and collected with high performance liquid chromatography (HPLC, Toyo, Tokyo). One part of the IgG fraction was used as the first coating ab and another was biotinylated with N-hydroxysuccinimidobiotin (Sigma) as the second ab biotin conjugate by the method of Bayer and Wilchek²⁵⁾. The other steps were the same as for the total IgA assay. The crude extract of *D. farinae* contained about 14 μ g/mg *Der f* I and 16 μ g/ml *Der f* II, respectively.

Determination of specific total IgA and SIgA ab to the crude extract of *D. farinae*, *Der f* I and *Der f* II: Allergen-specific IgA and SIgA abs were determined by a method similar to that for the total IgA assay. Each of three kinds of allergens were coated at a concentration of 10 μ g/ml. Since standard specific total IgA and SIgA ab to these allergens were not available, a reference sample was selected from the specimens of which ab activity was the strongest and the ab value in

dilution at $1:10^2$ of the sample was arbitrarily defined as 100 units/g secretion. Samples diluted at $1:3 \times 10^2$ were assayed and the ab values of each specimen were calculated from the standard curve of the reference sample. Goat antihuman IgA (α -chain specific) biotin conjugate (Sigma) diluted to $1:5 \times 10^4$ and rabbit antihuman secretory component (Dako) conjugated with N-hydroxysuccinimidobiotin (Sigma) according to the method mentioned above²⁵, diluted to 1 $\mu\text{g/ml}$, were used for specific total IgA and SIgA assays, respectively. The whole procedure was identical to that for the total IgA assay.

To test the specificity of the binding between coated allergens and IgA ab in the nasal secretions, the reference sample was pre-incubated with equal volumes of serial dilutions of the three types of allergens and similar concentration of BSA (concentrations for crude extract of *D. farinae* were from 1 to 100 $\mu\text{g/ml}$ and for *Der f* I and *Der f* II from 0.25 to 25 $\mu\text{g/ml}$), respectively at 4°C overnight. The mixtures were then examined for non-combined allergen-specific IgA ab by the assay described above. The crude extract of *D. farinae* (10 $\mu\text{g/ml}$) and *Der f* I and *Der f* II (5 $\mu\text{g/ml}$ each) almost completely inhibited specific binding, while BSA produced little inhibition. Dose-dependent inhibition was observed.

Determination of specific IgG ab to crude extract of *D. farinae*, *Der f* I and *Der f* II: The procedure for allergen-specific IgG ab assay was the same as that for the specific IgA ab assay. For the same reason as that indicated in the specific IgA assay, the antibody activity of a reference sample, diluted at 1:100, was arbitrarily designated as 100 units/g. Samples were assayed mostly at a dilution of 1:100, and a few samples with higher ab titers at a dilution of 1:300. Goat

antihuman IgG biotin conjugate (Sigma), diluted to $1:10^4$, was used. The subsequent steps in this assay were identical to those for the specific IgA assay. To confirm that the assays for IgG were specific, allergen inhibition tests were carried out, which were similar to the IgA specificity assay. The crude extract of *D. farinae* (10 $\mu\text{g/ml}$) and *Der f* I and *Der f* II (2.5 $\mu\text{g/ml}$ each) produced a nearly complete inhibition, and dose-dependent inhibition was also noted.

Determination of IgE and specific IgE ab to crude extract of *D. farinae* in serum: Enzyme-linked immunoassay (EIA) was used for IgE assay (EIA kit, Mitsui-II, Mitsui Pharmaceuticals INC), and RAST was applied for specific IgE ab assay with a Pharmacia RAST RIA kit.

Statistical analysis

Comparisons among the groups were made by Student's *t*-test when the values were normally distributed and by Wilcoxon test when the distributions were nonparametric. Correlations between the parameters were expressed by the Pearson and Spearman correlation coefficients for normally and nonparametrically distributed values, respectively.

RESULTS

Protein, albumin and immunoglobulins

The protein concentrations of the nasal secretions are listed in Table 1. The concentrations of total protein, total IgA, SIgA and IgG were similar in the normal group to the allergy groups. However, the normal group showed lower albumin ($p < 0.05$) and a higher ratio of SIgA/Total IgA ($p < 0.02$) than the allergy groups.

Table 1 Protein, albumin and immunoglobulins

	Weight (mg)	Protein (mg/g)	Albumin (mg/g)	Total IgA (mg/g)	SIgA (mg/g)	SIgA/T-IgA (%)	IgG ($\mu\text{g/g}$)
Normal subjects	41.9 $\pm$ 7.1 (4.6-383.3)	8.40 $\pm$ 1.4 (1.10-32.00)	0.75 $\pm$ 0.12 (0.02-8.36)	3.07 $\pm$ 0.63 (0.41-20.00)	2.38 $\pm$ 0.49 (0.34-15.00)	77.5 $\pm$ 1.6 (54.1-99.9)	960.7 $\pm$ 293.6 (25.0-9999.0)
Allergic patients (untreated)	86.5* $\pm$ 19.3 (15.8-412.9)	7.05 $\pm$ 1.6 (1.00-35.00)	1.24* $\pm$ 0.24 (0.20-8.36)	2.14 $\pm$ 0.42 (0.44-8.50)	1.53 $\pm$ 0.32 (0.34-6.75)	71.2* $\pm$ 1.6 (54.2-92.9)	806.1 $\pm$ 182.0 (83.0-2500.0)
Allergic patients (treated)	62.5* $\pm$ 23.2 (4.8-669.0)	9.03 $\pm$ 1.0 (1.60-20.50)	1.11* $\pm$ 0.20 (0.15-4.75)	3.28 $\pm$ 0.43 (0.30-9.75)	2.31 $\pm$ 0.29 (0.26-7.00)	71.9* $\pm$ 1.5 (50.0-90.8)	800.7 $\pm$ 170.4 (25.0-4000.0)

Results were expressed as mean $\pm$ SE.

* $p < 0.05$ when compared with normal subjects

Table 2 Correlation coefficients among components in nasal secretions

Correlation	Normal subjects		Allergic patients (untreated)		Allergic patients (treated)	
	R	P	R	P	R	P
Protein vs albumin	0.4140	0.018	0.3460	NS	0.4025	0.028
Protein vs SIgA	0.5520	0.001	0.4233	0.04	0.6603	0.0001
Protein vs IgG	0.5034	0.003	0.5155	0.001	0.5964	0.04
Albumin vs Total IgA	0.5428	0.0007	0.0486	NS	0.1922	NS
Albumin vs SIgA	0.5109	0.002	0.0190	NS	0.1822	NS
Albumin vs IgG	0.7023	0.0001	0.8412	0.0001	0.8845	0.0001
IgG vs SIgA	0.7613	0.0001	0.2335	NS	0.3229	NS
Total IgA vs SIgA	0.9953	0.0001	0.9911	0.0001	0.9876	0.0001

NS: No significant difference $p > 0.05$

Total protein concentrations correlated positively with the concentrations of albumin, IgA or SIgA and IgG in the normal group and allergy groups. The albumin concentrations correlated highly with IgG concentrations in the three groups but correlated with the concentrations of total IgA and SIgA only in the normal group. The concentrations of total IgA and SIgA correlated highly (Table 2).

Total IgA and SIgA antibodies

The allergy groups displayed much higher total IgA and SIgA abs against the crude extract of *D. farinae* than the normal group ($p < 0.0001$ between the normal group and the treated or untreated allergy groups), but no significant difference was found between the untreated and treated allergy groups (Fig. 1). Total IgA and SIgA abs to *Der f* I and *Der f* II were higher in the allergy groups (both untreated and treated) than in the normal group ($p < 0.004$ and $p < 0.008$), and the treated group showed slightly increased levels of total IgA and SIgA abs to *Der f* II ($p < 0.04$ and $p < 0.01$ respectively) than the untreated group, but not to *Der f* I (Fig. 2 and Fig. 3).

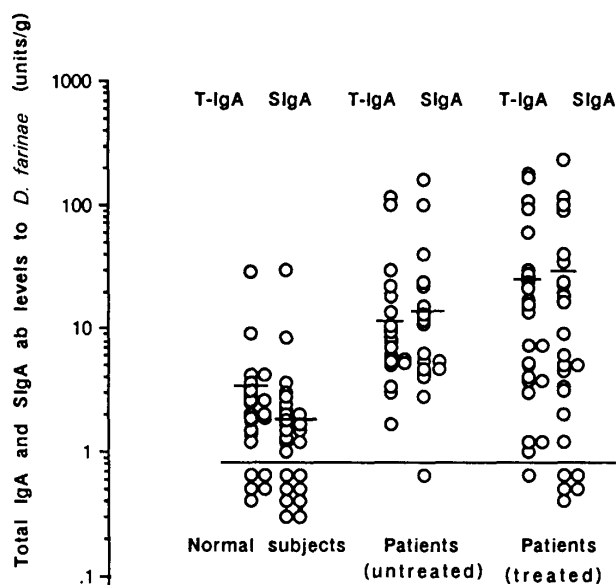


Fig. 1. Total IgA and SIgA ab levels to *D. farinae* in nasal secretion. Total IgA and SIgA in units/g secretion was measured by avidin-biotin ELISA in the normal group, untreated group and treated group. In Figs. 1 to 7, short solid lines indicate mean values of abs and subjects with undetectable abs are shown below long solid line which indicates the limit of detection.

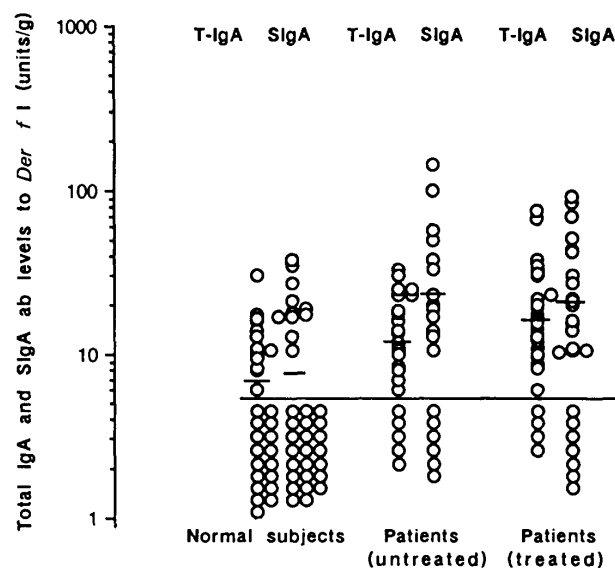


Fig. 2. Total IgA and SIgA ab levels to *Der f* I in nasal secretion. Allergic patients exhibit higher levels of IgA and SIgA abs than normal subjects.

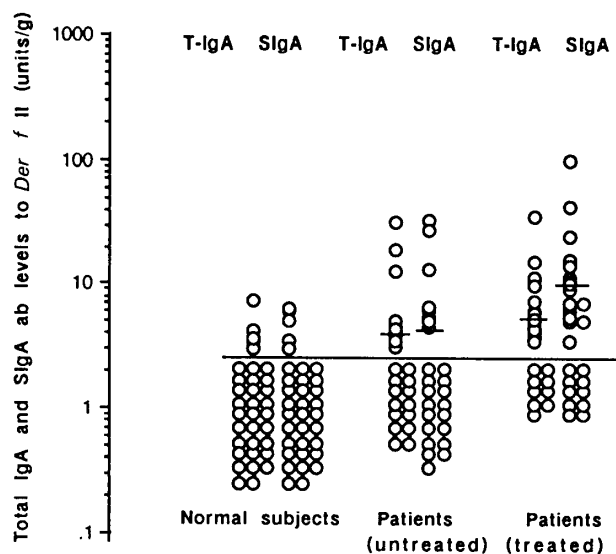


Fig. 3. Total IgA and SIgA ab levels to *Der f II* in nasal secretion. Treated allergic patients have slightly higher levels of abs than untreated patients, and untreated patients display higher levels of abs than normal subjects.

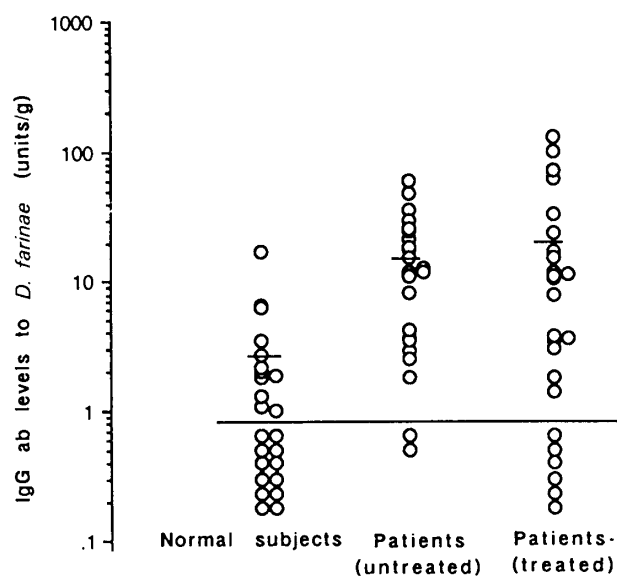


Fig. 4. IgG ab levels to *D. farinae* in nasal secretion. Allergic patients display higher levels of IgG abs than normal subjects.

IgG antibodies

The allergy groups (untreated and treated) showed higher levels of IgG abs against the crude extract of *D. farinae* ($p < 0.0001$ and $p < 0.002$), *Der f I* ($p < 0.004$ and $p < 0.004$) and *Der f II* ($p < 0.0001$ and $p < 0.0008$) than the normal group. The treated group had higher IgG ab levels than the untreated group, but the difference was not statistically

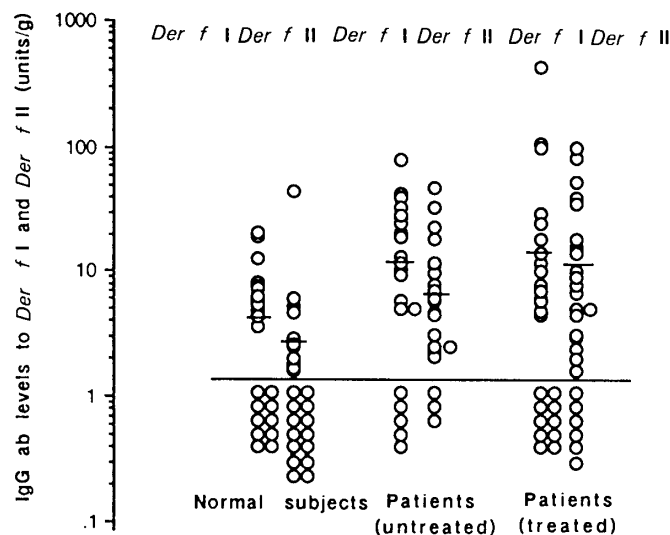


Fig. 5. IgG ab levels to *Der f I* and *Der f II* in nasal secretion. Allergic patients show higher IgG abs than normal subjects.

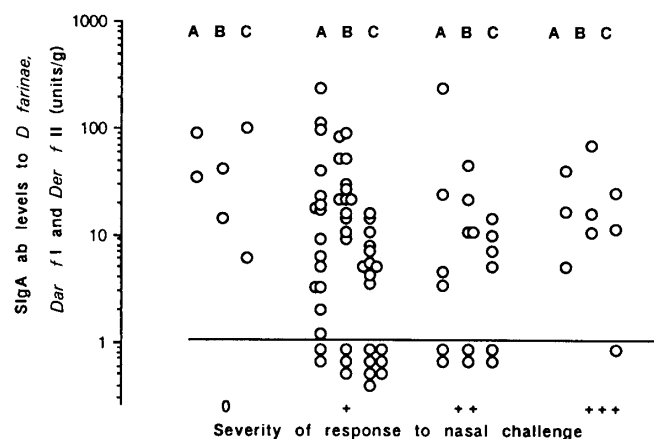


Fig. 6. Specific SIgA ab levels to mite allergens and the degree of response to nasal challenge in the treated allergy group. The severity of response is classified as 0, +, ++ and +++ respectively. A: abs to *D. farinae*, B: abs to *Der f I* and C: abs to *Der f II*. There is no obvious correlation between the levels of abs and the severity of response to nasal challenge.

significant (Fig. 4 and Fig. 5).

Relationship among abs of the same and different immunoglobulin (Ig) classes

Total IgA and SIgA to each of three types of allergens correlated closely with each other. Since *Der f I* and *Der f II* are the major allergens in the crude extract of *D. farinae*, it was expected that the levels of abs to each of three kinds of allergens might be closely related. It was found that total

Table 3 Correlation coefficients among specific IgA, IgG and IgE abs

Correlation	Normal subjects (n=28)		Allergic patients (n=25) (untreated)		Allergic patients (n=28) (treated)	
	R	P	R	P	R	P
Total IgA vs SIgA						
<i>D. farinae</i>	0.9922	0.0001	0.9825	0.0001	0.9913	0.0001
<i>Der f</i> I	0.7470	0.0001	0.7892	0.0001	0.8330	0.0001
<i>Der f</i> II	0.8444	0.0001	0.9270	0.0001	0.8403	0.0001
Total IgA						
<i>D. farinae</i> vs <i>Der f</i> I	0.2531	NS	0.7683	0.0001	0.5829	0.001
<i>D. farinae</i> vs <i>Der f</i> II	0.3243	NS	0.9012	0.0001	0.8182	0.0001
<i>Der f</i> I vs <i>Der f</i> II	NC		0.7908	0.0001	0.6340	0.0003
SIgA						
<i>D. farinae</i> vs <i>Der f</i> I	NC		0.6694	0.0002	0.6785	0.0001
<i>D. farinae</i> vs <i>Der f</i> II	NC		0.9012	0.0001	0.5318	0.004
<i>Der f</i> I vs <i>Der f</i> II	NC		0.6286	0.0007	0.5468	0.0026
IgG						
<i>D. farinae</i> vs <i>Der f</i> I	0.5462	0.004	0.6532	0.0004	0.7736	0.0001
<i>D. farinae</i> vs <i>Der f</i> II	0.9226	0.0001	0.5306	0.0064	0.5476	0.0003
<i>Der f</i> I vs <i>Der f</i> II	0.6175	0.0007	0.6879	0.0001	0.5618	0.003
SIgA vs IgG						
<i>D. farinae</i>	0.3449	NS	0.0563	NS	0.7422	0.0001
<i>Der f</i> I	NC		-0.2155	NS	0.7451	0.0001
<i>Der f</i> II	NC		0.1403	NS	0.5620	0.002
<i>D. farinae</i> SIgA vs IgE*	ND		-0.0735	NS	0.6487	0.0001
<i>D. farinae</i> SIgA vs IgE ab	ND		0.2343	NS	0.4880	0.01
<i>D. farinae</i> IgG vs IgE	ND		-0.1548	NS	0.6484	0.0003
<i>D. farinae</i> IgG vs IgE ab	ND		-0.1303	NS	0.4056	0.04

NC: not compared due to few number of subjects with detectable specific abs.

*IgE and IgE abs to *D. farinae* were determined only in serum.

NS: no significant difference $p > 0.05$.

ND: not done.

IgA and SIgA ab correlated positively between the crude extract of *D. farinae* and *Der f* I, between the crude extract of *D. farinae* and *Der f* II and between *Der f* I and *Der f* II in the treated and untreated allergy groups. Correlation analysis could not be carried out in the normal group since the abs to *Der f* I and *Der f* II were detected only in a few cases (Fig. 2 and Fig. 3). Similarly, IgG abs to the three allergens could also be related. It was confirmed that all three groups showed positive correlation. Abs to each of three types of allergens between SIgA and IgG abs correlated in the treated allergy group but not in the untreated allergy group. Furthermore these three kinds of abs in nasal secretions correlated with serum IgE and IgE ab to *D. farinae* in sera from the treated allergic group. The correlation coefficients are summarized in Table 3.

Table 4 The levels of specific IgA and IgG abs in clinically improved and unimproved patients

	Improved (n=13) (units, Mean $\pm$ SE)	Unimproved (n=15) (units, Mean $\pm$ SE)
<i>D. farinae</i>		
Total IgA	41.8 $\pm$ 15.1	19.9 $\pm$ 10.6
SIgA	51.4 $\pm$ 18.7	25.0 $\pm$ 15.1
IgG	25.7 $\pm$ 9.6	28.6 $\pm$ 14.7
<i>Der f</i> I		
Total IgA	19.6 $\pm$ 2.4	20.6 $\pm$ 6.1
SIgA	29.6 $\pm$ 6.3	21.1 $\pm$ 7.1
IgG	27.3 $\pm$ 10.6	35.6 $\pm$ 30.5
<i>Der f</i> II		
Total IgA	7.5 $\pm$ 2.5	3.9 $\pm$ 1.2
SIgA	16.9 $\pm$ 7.5	5.7 $\pm$ 1.9
IgG	23.0 $\pm$ 9.8	106 $\pm$ 4.3

No significant difference was found between the two groups.

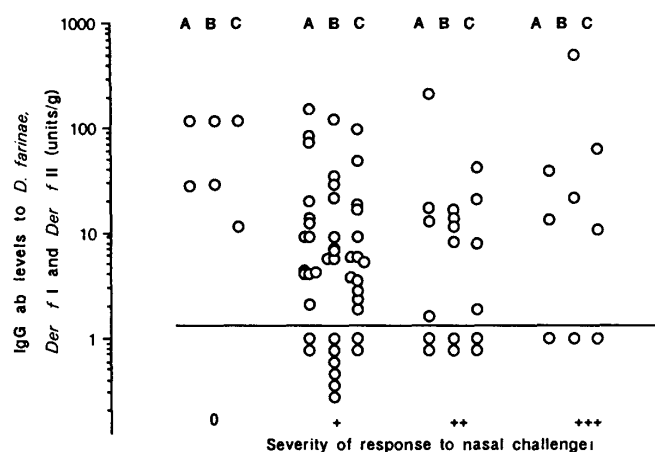


Fig. 7. Specific IgG ab levels to mite allergens and the degree of response to nasal challenge in the treated allergy group. A: abs to *D. farinae*, B: abs to *Der f I* and C: abs to *Der f II*. There is no correlation between the two parameters.

IgA and IgG ab levels versus clinical improvement

Twenty-eight treated patients were divided into two groups according to the effect of immunotherapy on the symptoms and reactions to allergen challenge. It seemed that the clinically improved group showed a higher IgA ab level than the unimproved group, but the difference was not significant (Table 4), and the level of abs did not correlate with the degree of response to nasal challenge (Fig. 6 and 7).

IgA and IgG abs in nasal secretions and in sera

The ratio of the specific IgA or IgG abs per each class of immunoglobulin in nasal secretions to those in sera was much higher for IgA abs but equal for IgG abs (Fig. 8).

IgE and IgE ab in sera

The concentrations of IgE in sera were $1255 \pm$

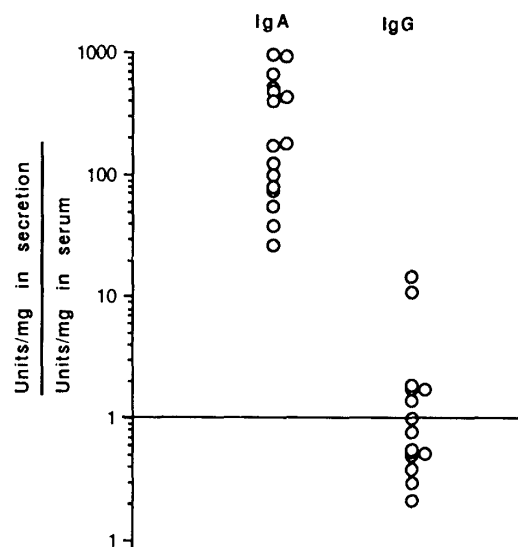


Fig. 8. The ratios of specific ab levels in secretion and in serum to *D. farinae*. Sixteen samples from the untreated allergy group were shown.

358 and 696 ± 143 IU/ml (mean $\pm$ SE) for the untreated and treated groups, respectively. The levels of specific IgE ab to the crude extract of *D. farinae* was expressed as a ratio of the RAST value in the sample examined to that in reference serum A provided in the kit (%): about $99.3 \pm 7.3\%$ and $87.9 \pm 9.8\%$ for the untreated and treated groups, respectively.

IgA and IgG abs in saliva

It was found that the concentrations of protein components in whole unstimulated saliva were much lower than those in nasal secretions. Total IgA and SIgA concentrations were about 10 times and albumin and IgG concentrations were about 30 to 40 times lower than those in nasal secretions. Almost all the IgA in saliva was SIgA. When the saliva were examined at a dilution of $1:10^2$ (a

Table 5 Albumin, IgA, IgG and SIgA abs to *D. farinae* mite allergens in saliva

	Albumin ($\mu\text{g/g}$)	Total IgA ($\mu\text{g/g}$)	SIgA ($\mu\text{g/g}$)	SIgA/T-IgA (%)	IgG ($\mu\text{g/g}$)	Number of subjects with detectable SIgA ab to		
						<i>D. farinae</i>	<i>Der f I</i>	<i>Der f II</i>
Normal subjects	19.5 ± 5.7 (2.0-50.0)	188.8 ± 44.0 (30.0-360.0)	186.3 ± 43.7 (30.0-370.0)	96.1 ± 1.4 (89.3-100.0)	11.0 ± 3.7 (1.0-28.0)	2*/8	2/8	1/8
Allergic patients (untreated)	28.7 ± 5.2 (15.0-48.0)	311.4 ± 67.8 (140.0-590.0)	332.8 ± 99.3 (120.0-880.0)	93.2 ± 2.6 (81.8-100.0)	16.1 ± 3.2 (5.0-27.0)	1/8	1/8	1/8
Allergic patients (treated)	37.5 ± 6.6 (3.0-74.0)	308.0 ± 64.3 (80.0-800.0)	262.0 ± 44.3 (70.0-590.0)	89.2 ± 3.1 (74.1-100.0)	21.0 ± 5.0 (1.0-60.0)	4/11	2/9	1/9

*The number of subjects containing detectable specific abs to *D. farinae* mite allergens in their salivas.

few cases at 1:10), the concentration of detectable specific abs were very low. Seven out of 27, 5 or 3 out of 25 subjects examined showed detectable IgA or SIgA ab to crude extract of *D. farinae*, *Der f I* or *Der f II*, respectively, while only one subject showed detectable IgG ab to *Der f I* (Table 5). It was demonstrate that the whole unstimulated saliva also contained SIgA ab to *D. farinae* mite allergens although the concentrations of the ab were at much lower level than nasal secretion.

DISCUSSION

We measured the concentrations of protein, albumin, total IgA, SIgA, IgG and SIgA and IgG abs against crude extract of *D. farinae* and its purified allergens *Der f I* and *Der f II* in nasal secretions by an up-to-date sensitive method of avidin-biotin ELISA. This method increased the sensitivity of measurement so that trace amounts of allergen-specific abs could be detected even in a few normal subjects. The method also enabled us to express the concentrations as absolute values but not as relative values such as ab binding activity/IgA^(6,7) or ab/albumin^(8,10,11,16).

The allergy groups showed higher concentrations of albumin but a lower SIgA/Total IgA ratio (Table 1) than the normal group, which might be explained by an increased permeability of the mucosal blood vessels caused by allergic inflammation. The concentrations of albumin and IgG correlated closely (Table 2) in the three groups. This implies that they might be have the same origin (from serum). The present study has confirmed that SIgA and IgG ab levels in nasal secretions are higher in allergic patients than in normal subjects, as found in previous studies^(15,16). The results from the purified allergens were similar to those from the crude extract of *D. farinae*. This suggests that allergic patients possibly have an increased activity to produce specific SIgA and IgG abs to mite as well as specific IgE ab, or that they have more natural exposure to mite than normal subjects. Specific SIgA and IgG abs to mite allergen in nasal secretions seemed to be insufficient to inhibit allergic reactions, as Plattis-Mills et al⁽⁶⁾, Johansson et al⁽¹¹⁾ and Okuda⁽¹⁴⁾ have already previously reported in connection

with pollinosis or mite nasal allergy, since there was no significant correlation between the amount of specific SIgA and IgG abs in nasal secretions and nasal symptoms or decreased sensitivity to nasal challenges.

Parenteral immunotherapy increased IgG ab levels in sera^(19,26) but not in nasal secretions as documented in previous reports^(14,15,27). In our study, there was no correlation concerning SIgA and IgG abs between the treated and untreated groups or between the groups improved or unimproved by immunotherapy. Parenteral immunotherapy may not promote well local immunity to inhalant allergens since the nose associated lymphoid tissue is not directly stimulated by parenteral immunotherapy.

We could not reach any conclusion as to which of the two *Der f I* or *Der f II* is more important in inducing local production of specific SIgA ab, because it is impossible to compare them directly due to the lack of standard specific abs common to both purified allergens. However, the ab to *Der f II* was undetectable more often than that to *Der f I* in the present study.

We also confirmed the close correlations among abs of the same classes of Ig in all three groups and among abs of different classes of Ig in the treated allergy group, but not in the untreated group (Table 3). IgE and IgE ab levels in sera also correlated with IgA, SIgA and IgG ab levels in nasal secretions in the treated group. We did not determine IgE ab levels in nasal secretions in this study, but the levels in sera and in secretions correlated well with each other⁽²⁸⁾. Immunotherapy might be responsible for this difference. However, we could not clearly identity the reason for the difference on the basis of our results among the treated and untreated groups.

Concerning the source of each specific abs, it is undoubtful that SIgA ab is produced locally as proposed previously^(7,14), since the ratio of IgA ab in secretions to sera was very high and there was no correlation between SIgA and albumin in the allergic patients. On the other hand, specific IgG ab may come from the circulation through transudation, since the ratio of IgG abs in secretions to sera was very low and the concentrations of IgG and albumin were closely correlated.

Detection of specific SIgA ab to crude extract of

D. farinae, *Der f I* and *Der f II* in saliva seems to support the idea that local production of specific IgA ab is part of a generalized secretory response called the common mucosa immune system²⁹⁾. On the other hand, the concentration of SIgA ab was much higher in nasal secretions than in saliva, suggesting that local stimulation with allergen potentiates local production of abs.

In conclusion, normal subjects and allergic patients have the ability to respond to the environmental allergens *Der f I* and *Der f II* as well as to crude mite allergen and produce allergen-specific IgA and IgG abs. However allergic patients produced higher levels of IgA and IgG abs than the normal subjects. The concentration of abs to allergens correlates well among the same and different Ig classes, and parenteral immunotherapy has little influence on the local production of the ab.

We would like to thank Dr. Andoh, research laboratories of Torii Co. LTD., Ichikawa, Japan, for kindly providing us purified allergens *Der f I* and *Der f II* and antisera to the two allergens

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鼻汁中抗コナヒョウヒダニ特異的 IgA, IgG 抗体の研究

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鼻汁中抗精製ダニ *Der f* I, II 特異的抗体量の報告は我々の知る限りない。そこで、我々は吸引により鼻汁を採取し、粗および精製ダニ抗原 (*Der f* I, II) に対する IgA, SIgA, IgG 抗体を34人の非アレルギー、25人の未治療、28人の既治療(免疫療法)ダニ鼻アレルギー患者を対象に、高感度の avidin-biotin ELISA 法で定量した。

その結果は次のようであった。1. いずれの抗原に対するいずれの抗体も、互いに相関して、アレルギー患者では非アレルギー者に比し増加していた。2. これら鼻汁中特異抗体はダニ抗原による鼻反応に影響を与えなかった。3. 免疫療法はこれら鼻汁中抗体量を有意に増加させなかった。4. 鼻汁中 IgA, SIgA 抗体は局所で産生され、IgG 抗体は血管からの漏出によると考えられた。