



Immunization of Rabbits with Nematode *Ascaris lumbricoides* Antigens Induces Antibodies Cross-Reactive to House Dust Mite *Dermatophagoides farinae* Antigens

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There are controversial reports on the relationship between helminthic infection and allergic diseases. Although IgE cross-reactivity between nematode *Ascaris* antigens and house dust-mite allergens in allergic patients have been reported, whether *Ascaris* or the mite is the primary sensitizer remains unknown. Here we found that immunization of naïve animals with *Ascaris lumbricoides* (Al) antigens induced production of antibodies cross-reactive to mite antigens from *Dermatophagoides farinae* (Df). Sera from Bangladeshi children showed IgE reactivity to *Ascaris* and mite extracts. IgG from rabbits immunized with Al extract exhibited reactivity to Df antigens. Treatment of the anti-Al antibody with Df antigen-coupled beads eliminated the reactivity to Df antigens. In immunoblot analysis, an approximately 100-kDa Df band was the most reactive to anti-Al IgG. The present study is the first step towards the establishment of animal models to study the relationship between *Ascaris* infection and mite-induced allergic diseases.

Key words: *Ascaris lumbricoides*; *Dermatophagoides farinae*; house dust mite; cross-reactivity; rabbit antiserum

It is estimated that 1.5 billion people are infected with intestinal parasites around the world¹⁾ and that a similar number of the patients suffer from allergic diseases.²⁾ There are controversial reports on the relationship between helminthic infection and allergic diseases. Some of them suggest that helminthic infection promotes allergic disease,³⁻⁷⁾ while others suggest that helminthic infection against allergies has a protective role against allergic diseases.⁸⁻¹¹⁾

Recent reports describe IgE cross-reactivity between nematode *Ascaris* and house-dust mite (HDM) allergens in allergic patients, suggesting that *Ascaris* or HDMs might be a primary sensitizer promoting or modulating sensitization to each other.¹²⁻¹⁵⁾ Here we examined to determine whether immunization of naïve animals with *Ascaris* antigens can induce the production of antibodies cross-reactive to HDM antigens from *Dermatophagoides farinae* (Df).

Materials and Methods

Ascaris, HDM, and cockroach extracts. Nematodes, *Ascaris lumbricoides* (Al), were collected from *Ascaris*-infected patients at the International Center for Diarrheal Disease Research in Bangladesh. The study complied with the Code of Ethics of the World Medical Association (The Helsinki Declaration of 1964, as revised in 2002). The nematodes were frozen and sent to the Clinical Research Center for Allergy and Rheumatology, National Hospital Organization at Sagamiara National Hospital in Japan. Two female nematodes were lyophilized and crushed with a mortar and a pestle. Dried powder (0.6 g) was defatted with ethyl ether (30 mL) and reconstituted in 125 mM ammonium bicarbonate (12 mL). After centrifugation (30,000 g, 10 min), the supernatant was collected and dialyzed against 5 mM ammonium bicarbonate.

Mites were isolated from whole cultures of Df. Isolated mite bodies (4.0 g) were defatted, crushed with a mortar and pestle, and incubated in 125 mM ammonium bicarbonate (200 mL, 1:50 w/v) at 4 °C overnight. After centrifugation (30,000 g, 20 min), 150 mL of the supernatant was dialyzed against 5 mM ammonium bicarbonate (*D. farinae* A). Commercial mite body extracts of Df and Dp (LSL, Tokyo) were also used (*D. farinae* B and *D. pteronyssinus* B). Whole Df culture extracts¹⁶⁾ were used to make Df extract-coupled sepharose beads to remove antibodies cross-reactive to Df antigens.

German cockroach, *Blattella germanica* (Bg), extract (Greer, Lenoir, NC, USA) was defatted and lyophilized. The defatted powder from cockroaches was reconstituted in 125 mM ammonium bicarbonate.

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Abbreviations: Al, *Ascaris lumbricoides*; Bg, *Blattella germanica*; CBB, Coomassie Brilliant Blue; Df, *Dermatophagoides farinae*; Dp, *Dermatophagoides pteronyssinus*; HDM, house-dust mite

After centrifugation, the supernatant was dialyzed against 5 mM ammonium bicarbonate. Dialyzed extracts of Al, Df, Dp, and Bg were lyophilized and stored at 4 °C until use.

Protein concentration. Protein concentrations were measured by the DC protein assay (Bio-Rad, Hercules, CA, USA).

Immunization of rabbits with *Ascaris lumbricoides* extract and purification of IgG from rabbit anti-*Ascaris* antisera. The protocol was approved by a committee of Takara Bio (Ohtsu, Japan), and immunization was carried out in line with the Guidelines for the Care and Use of Laboratory Animals in Takara Bio. Antisera to Al were prepared by immunizing rabbits with Al extract with Freund's complete adjuvant. Two 12-month-old rabbits were injected subcutaneously with 0.4 mg of Al extract, resolved in PBS and mixed with the adjuvant, at weekly intervals for 4 weeks, and were bled out 10 d after the last injection. The rabbits were housed in cages isolated from other allergens, *e.g.*, mites and pollen, at Takara Bio. Control serum from a rabbit immunized with an unrelated peptide conjugated with the same adjuvant was the kind gift of Takara Bio.

IgG antibodies in the antisera were purified with a Hi-Trap protein A HP column (GE Healthcare, Little Chalfont, UK) and were dialyzed with PBS containing 0.02% sodium azide. Then, they were stored at -30 °C until use.

Removal of antibodies cross-reactive with *D. farinae* antigens. To remove HDM antigen-reactive antibodies, Df extract-coupled sepharose beads were prepared. One g of CNBr-activated sepharose 4B (GE Healthcare) was washed with 1 mM HCl. Twenty-five mg of whole Df mite culture extract was reconstituted in 5 mL of a coupling buffer (0.1 M NaHCO₃ pH 8.3 and 0.5 M NaCl). Activated sepharose and Df extract were mixed and the mixture was incubated for 1 h at room temperature. Five mL of 1 M ethanolamine (pH 8.0) was added to block excess groups after washing with coupling buffer. Df-coupled sepharose 4B was washed with 0.1 M acetate buffer (pH 3.5) and 0.1 M Tris-HCl buffer (pH 8.0) containing 0.5 M NaCl by turns. It was then washed and suspended with PBS containing 0.02% sodium azide.

IgG antibodies reactive to Df antigens were removed by incubation with Df-coupled sepharose 4B. Four mL of Df-coupled sepharose 4B, 2.5 mL of PBS, and 1.5 mL of rabbit IgG (2.3 mg/mL) were mixed and incubated for 1 h at room temperature. The supernatant was collected by filtration, and 4 mL of purified antibody (0.56 mg/mL) was obtained. To regenerate Df-coupled sepharose 4B, 0.1 M acetate buffer containing 0.5 M NaCl was added and the mixture was rotated for 15 min to remove Df-reactive antibodies from the sepharose 4B, followed by PBS washing. The regenerated Df-coupled sepharose 4B was reused in the removal of Df-reactive antibodies. In the end, 4.5 mg of purified IgG was obtained.

Measurement of human anti-*Ascaris* IgE. Sera were collected from children who had specific IgE to *Ascaris* in a cohort study in Matlab, Bangladesh.³⁾ This study was approved by the Ethical Review Committee of the International Center for Diarrheal Disease Research, Bangladesh. A paper disk assay was carried out to determine the levels of Al-specific IgE antibodies, as previously described.^{17,18)} Briefly, *Ascaris* extract prepared as described above was coupled to CNBr-activated disks. The Al-coupled disks were incubated with sera. After washing, β -galactosidase-conjugated anti-human IgE (Phadia, Uppsala, Sweden) was added. After incubation overnight and washing, the activity of β -galactosidase was measured. The assay was calibrated using a control curve obtained with disks coupled with Japanese cedar pollen extracts and serial dilutions of pooled sera from patients with an allergy to Japanese cedar pollen that had been substandardized against the Phadia CAP system. Results were extrapolated from the control curve, and were expressed as unit/mL. Hence the IgE antibody levels determined by ELISA were assumed to be approximately equivalent to those determined by the CAP system (KU_A/L).

Immunoblot analysis of the binding of human anti-*Ascaris* IgE. Lyophilized Al preparations (15 μ g/lane) were subjected to 4–20% SDS-PAGE (Tefco, Tokyo) and electroblotted onto nitrocellulose membranes. Broad-range molecular markers (Bio-Rad) were used as standards to estimate apparent molecular weights. After blocking with PBS containing 1% BSA, the membrane was incubated in 1:20 patient

serum diluted with PBS containing 0.1% BSA and 0.05% Tween 20 for 1 h at room temperature. After washing, the membrane was incubated with a 1:3,000 dilution of horseradish-peroxidase-conjugated goat anti-human IgE (Invitrogen, Carlsbad, CA, USA) for 1 h at room temperature. Peroxidase activity was visualized by the Amersham ECL Western Blotting Analysis System (GE Healthcare) combined with Amersham Hyper Film ECL (GE Healthcare).

Immunoblot analysis of the binding of rabbit anti-*Ascaris* IgG. Al, Df, Dp, and Bg preparations (14 μ g/lane for Coomassie Brilliant Blue (CBB) staining, and 7 μ g/lane for immunoblotting) were electrophoretically separated on a 4–20% and on a 4–12% SDS-PAGE system. After blocking with PBS containing 1% BSA, the membranes were incubated in anti-Al antibody (50 ng/mL diluted with PBS containing 0.1% Tween 20 and 0.1% BSA) for 1 h at room temperature. Anti-Al antibodies were detected using a 1:200,000 dilution of peroxidase-conjugated goat F(ab')₂ anti-rabbit IgG (Invitrogen) after incubation for 1 h at room temperature. Peroxidase activity was visualized by the Amersham ECL Western Blotting Analysis System combined with Amersham Hyper Film ECL.

Fluorometric ELISA for binding of anti-*Ascaris* rabbit IgG. To detect cross-reactivity to the Al antigen, sandwich ELISA was used. Purified rabbit anti-*Ascaris* IgG was used as the capture antibody, and biotinylated rabbit anti-*Ascaris* IgG as the detection antibody. The capture antibody, diluted to 5 μ g/mL with bicarbonate buffer pH 9.6, was added to a C96 white maxisorp fluoronunc cert plate (Fisher Scientific, Roskilde, Denmark) (100 μ L/well) and incubated for 3 h at 37 °C. After washing with 0.9% saline containing 0.1% Tween 20 (250 μ L/well, 3 times), the plates were blocked with PBS containing 1% BSA for 1 h. After washing (250 μ L/well, 3 times), 100- μ L each of the samples and the standards were added to the wells, and the mixture was incubated for 4 h at 25 °C. After washing (250 μ L/well, 5 times), 100 ng/mL of the detection antibody diluted with PBS containing 10% normal rabbit serum was added (100 μ L/well) and the mixture was incubated overnight at 25 °C. After washing (250 μ L/well, 5 times), avidin-conjugated β -galactosidase was added (100 μ L/well), and the mixture was incubated for 1.5 h at 25 °C. After washing (250 μ L/well $\times$ 5), 0.1 mM 4-methylumbelliferyl- β -D-galactopyranoside in 0.02 M sodium phosphate buffer (pH 7.2) containing 0.1 M NaCl, 1 mM MgCl₂, 0.2% BSA, and 0.05% sodium azide (100 μ L/well) was added (100 μ L/well), and the mixture was incubated for 1 h at 37 °C. Glycine-NaOH (0.1 M, pH 10.4), (100 μ L/well) was added to stop the enzyme reactions. The fluorescence intensity was measured using a micro-plate fluorescence reader, Spectra Fluor (Tecan Group, Männedorf, Switzerland), at an excitation wavelength of 360 nm and an emission wavelength of 465 nm.

ELISAs for booklice, mosquitoes, moths, midges, and flies were performed by the same procedure. Antigens of mosquitoes, moths, midges, and flies were obtained as commercial products (Greer). Booklice were kindly donated by Dr. Yuji Kawakami of the FCG Research Institute. Isolated bodies were defatted, crushed by a mortar and pestle, and incubated in 125 mM ammonium bicarbonate at 4 °C overnight. After centrifugation, the supernatant was dialyzed against 5 mM ammonium bicarbonate and lyophilized. The lyophilized extracts were stored at 4 °C until use.

Results

Preparation of Ascaris lumbricoides extract

Ascaris extract was prepared from nematodes from *Ascaris*-infected individuals. The weight of dry power extract obtained from two female nematodes was 69 mg. In SDS-PAGE analysis of the *Ascaris* extract, many protein bands were detected (Fig. 1A). A band at 10 kDa was the most intensely stained band both under reducing and non-reducing conditions.

Human IgE-binding to Ascaris lumbricoides extract

To confirm the allergenicity in *Ascaris* extract we prepared, we analyzed its IgE reactivity using human

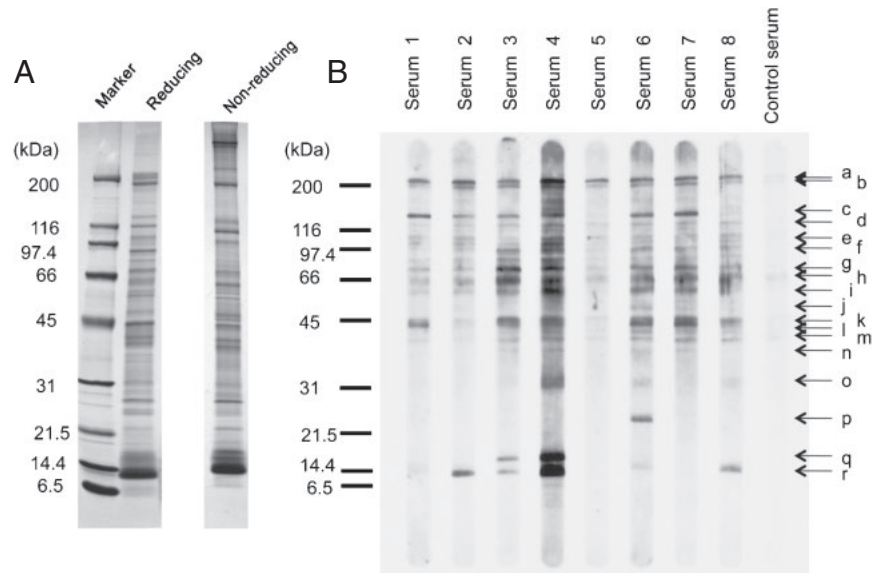


Fig. 1. Preparation and Human IgE-Binding of *Ascaris lumbricoides* Extract.

(A) CBB staining after SDS-PAGE (4–20%). (B) Immunoblot analysis. Human IgE-binding was analyzed in eight asthma Bangladeshi patients who had high titers of IgE specific to *Ascaris lumbricoides*. Bands mentioned in the text are indicated by arrows a to r. The data shown are representative of three independent experiments with similar results.

Table 1. IgE Titers Specific to *Ascaris lumbricoides*, *Dermatophagoides farinae*, and *Dermatophagoides pteronyssinus* Antigens in the Paper Disk Assay and the Phadia CAP System

Human IgE-binding was analyzed in eight asthma patients who had high titers of IgE specific to *A. lumbricoides*. Values are indicated in unit/mL in the paper disk assay and kU_A/L in the Phadia CAP system. In the Phadia CAP system, values higher than 0.34 kU_A/L were considered to be positive for anti-*Ascaris* IgE, and the maximum detection limit was 110 kU_A/L.

Serum number	1	2	3	4	5	6	7	8
<i>A. lumbricoides</i> (paper disk)	175.6	11.7	70.7	101	37	87.7	28.9	36.4
<i>A. lumbricoides</i> (CAP)	>110	>110	>110	>110	59.6	52	50.2	52.9
<i>D. pteronyssinus</i> (CAP)	13.7	39.1	0.34	0.51	110	0.58	31.6	1.03
<i>D. farinae</i> (CAP)	3.79	15.3	0.39	1.43	47.5	2.54	15.9	0.92

sera (Fig. 1B and Table 1). At least 18 bands were detected by immunoblot using sera of Bangladeshi children who had high IgE titers to Al (Fig. 1B, a–r). IgE binding to 10 bands, a–i and k, was common to all the sera, but that to bands j and l–r was not. For example, 6 sera out of 8 (sera 1–4, 6, and 8) showed IgE-binding to band r, and only one serum (serum 6) showed IgE-binding to band p.

Specific IgE reactivity to *Ascaris* was parallel between the paper disk assay using our *Ascaris* extract and the Phadia CAP system, which is widely used for diagnosis in clinic, in all the sera except for serum 2, in which the value in the paper disk assay was low although it was high in the Phadia CAP system (Table 1). The specific IgE reactivity to Dp correlated to that to Df in each of the sera. Since these results indicated that the *Ascaris* extract we prepared retained IgE reactivity, we used it in the immunization of the rabbits.

Binding of rabbit antibody in antisera against *Ascaris lumbricoides* extract to *A. lumbricoides*, *HDM*, and *cockroach* extracts

Sera were collected from rabbits immunized with Al extract. *Ascaris* (Al), HDMs (Df and Dp), and cockroach (Bg) extracts were subjected to fluorometric sandwich ELISA using anti-Al polyclonal antibodies (Fig. 2A). The IgG in the rabbit anti-Al sera showed the highest

reactivity to Al, moderate reactivity to Df, and no reactivity to Dp or Bg in the sandwich ELISA. Both the Df extract prepared in our laboratory and a commercial one, *D. farinae* A and B respectively (Fig. 2A) showed reactivity.

Rabbit IgG binding to many components in the Al extract and to some components in the Df extract was detected in immunoblot analysis (Fig. 2B). Rabbit IgG-binding to at least four components in the Df extract was detected in immunoblot analysis (Fig. 2B, left, bands s–v). No bands were detected with the use of the control serum (Fig. 2B, right).

Removal of antibodies cross-reactive to *D. farinae* antigens from rabbit anti-*Ascaris* antibodies

The rabbit IgG fraction from the anti-Al sera showed cross-reactivity with Df (Fig. 2 and Fig. 3A), and this was eliminated by absorption using Df-coupled sepharose 4B (Fig. 3B). We detected rabbit IgG-binding components in the *D. farinae* A (Fig. 3C, bands s, u, w, and x) and B extracts (Fig. 2A and Fig. 3C, bands s–v), but not in the Dp or the Bg extract, using the rabbit IgG fraction from anti-Al sera in immunoblot analysis (Fig. 3C, left). A protein band for approximately 100 kDa (Fig. 2B and Fig. 3C, u) showed the most significant reactivity. No bands were detected using sera after absorption with Df-coupled sepharose 4B (Fig. 3C, right).

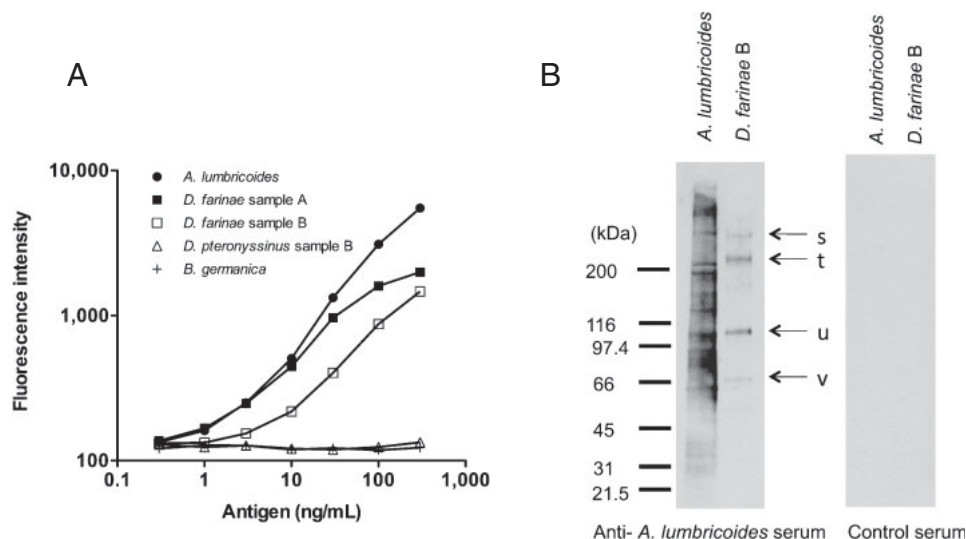


Fig. 2. Binding of IgG from Rabbits Immunized with *Ascaris lumbricoides* Extract to *Ascaris lumbricoides* and *D. farinae* Antigens.

(A) Sandwich ELISA using rabbit anti-*Ascaris* IgG. The data shown are means for two wells, and error bars indicate values for the two wells. (B) Immunoblotting after SDS-PAGE (4–20%) was carried out using rabbit anti-*Ascaris* serum (left) and control serum (right). Rabbit IgG was detected with enzyme-linked anti-rabbit IgG antibody. Control serum was collected from a rabbit immunized with an unrelated antigen mixed with the same adjuvant. Bands s to v were detected in immunoblot analysis using *D. farinae* B extract. The data shown are representative of three independent experiments.

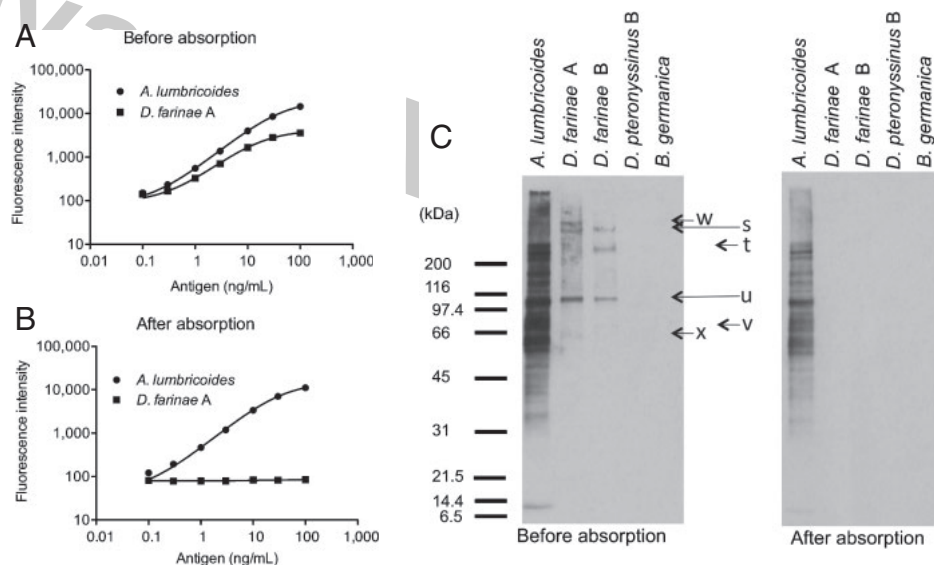


Fig. 3. Cross-Reactivity of IgG from Rabbits Immunized with *Ascaris lumbricoides* Extract to *D. farinae* Antigens.

Sandwich ELISA using rabbit anti-*Ascaris* IgG before (A) and after (B) absorption with Df-coupled sepharose 4B. (C) Binding to *Ascaris*, mite, and cockroach antigens analyzed by immunoblotting after SDS-PAGE (4–20%) using rabbit anti-*Ascaris* IgG before and after absorption. Bands w to z and s to v were detected in immunoblot analysis using rabbit anti-*Ascaris* IgG to *A. lumbricoides*, *D. farinae* A, and *D. farinae* B extracts respectively (arrows). The data shown are representative of four independent experiments. The data shown in A and B are the means for two wells, and error bars indicate values for the two wells.

The rabbit IgG fraction from anti-AI sera exhibited no cross-reactivity to invertebrates other than HDMs (cockroach, mosquito, fly, midge, booklice, and moth) before or after absorption with Df-coupled sepharose 4B (Fig. 4AB).

Discussion

Allergic diseases are now common even in under-developed countries in urban areas,¹²⁾ in which *Ascaris* infections frequently occur. Although a relationship between helminthic infection and allergic diseases has been suspected, whether and how they promote or suppress each other remains controversial.^{4–11)} Recently,

cross-reactivity between *Ascaris* and HDM antigens the latter of which are a major causative factor in allergic diseases such as asthma, rhinitis, conjunctivitis, and atopic dermatitis, has been reported.^{12–15)} In the present study, we immunized rabbits with *Ascaris* antigens and analyzed the cross-reactivity of the anti-*Ascaris* antibody to HDM antigens. Although extract of pig parasitic *Ascaris suum* is frequently used in research, the *Ascaris* extract used in the present study was prepared from *A. lumbricoides* (AI) parasitic on human. The sera from children with high titers of anti-AI IgE showed IgE reactivity to Df and Dp antigens (Fig. 1 and Table 1). Rabbit anti-AI IgG exhibited reactivity to Df antigens, but unexpectedly showed little or no reactivity to Dp

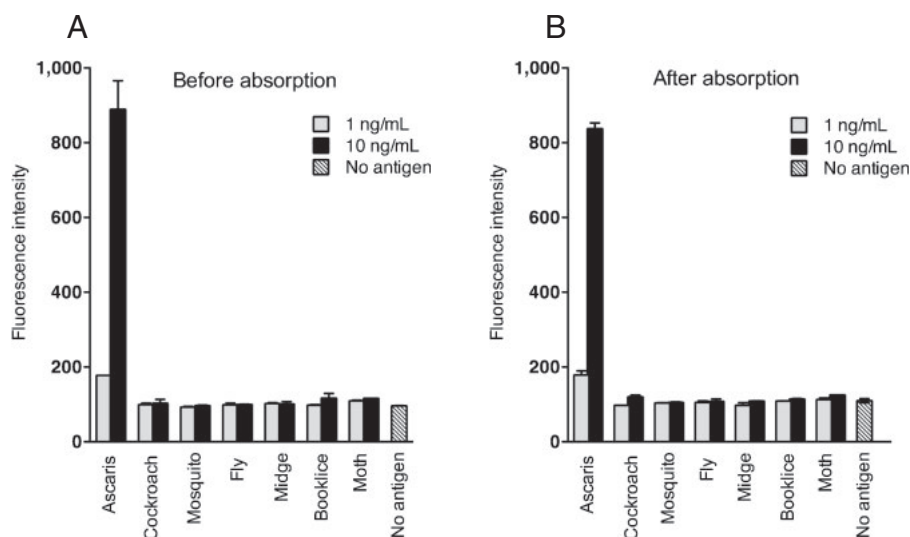


Fig. 4. The IgG from Rabbits Immunized with *Ascaris lumbricoides* Extract Showed No Cross-Reactivity with Other Invertebrates on Sandwich ELISA.

Fluorescence intensity was measured for each extract at 1 ng/mL and 10 ng/mL using IgG antibodies before absorption (A) and after absorption with *D. farinae*-coupled sepharose 4B (B). In A and B, the data shown are the means for two wells, and error bars indicate values for the two wells.

antigens (Figs. 2 and 3) or to cockroaches and other invertebrate antigens (Figs. 2A, 3C, and 4). An approximately 100 kDa Df band was the most reactive to anti-AI IgG (Figs. 2 and 3). As far as we know, this is the first demonstration that immunization of naïve animals with *Ascaris* antigens induces the production of antibodies cross-reactive to HDM antigens.

In immunoblot analysis using sera from Bangladeshi children who showed high IgE titers to AI antigens³⁾ at least 18 IgE-reactive bands were detected (Fig. 1B). The 10-kDa AI antigen was the most prominent protein component in CBB staining (Fig. 1A). The 10-kDa AI antigen band was reactive to IgE from six of eight sera (Fig. 1B, band r, sera 1–5, 6, and 8). Acevedo *et al.*¹⁴⁾ indicated that specific IgE to ABA-1 (Asc s 1), an allergen from another species of *Ascaris*, *A. suum*, a parasitic nematode that causes ascariasis in pigs, with a similar molecular weight, might be a specific marker of human *Ascaris* infection. We considered the component to be a homolog of ABA-1 in *A. lumbricoides* on the basis of its content in the extract, molecular weight, and IgE reactivity (Fig. 1). Thus, sera from children with high IgE titers to AI antigens showed IgE reactivity to HDM Df and Dp antigens, and the AI antigens we prepared showed similarity to the *A. suum* antigens.

The IgG in the rabbit antisera against AI showed the highest reactivity to AI and moderate reactivity to Df in a sandwich ELISA system, in which purified rabbit anti-*Ascaris* IgG was used as the capture antibody and a biotinylated one was used as the detection antibody (Figs. 2A and 3AB). In immunoblot analysis, the most reactive band in Df to rabbit anti-*Ascaris* IgG was at a molecular weight of approximately 100 kDa (Figs. 2B and 3C, u). HDM group 11 allergen paramyosin has a molecular weight of 98 kDa,¹⁹⁾ but further analysis is necessary to determine whether the protein for the band is the HDM paramyosin. A major allergen of another nematode, *Anisakis simplex*, Ani s 2, is also paramyosin.²⁰⁾ Although no experimental data suggested that

paramyosin is important as a cross-reactive antigen between *Ascaris* and HDM antigens until recently, a very recent report states that the recombinant Blo t 11 fusion protein showed IgE reactivity in 80% of allergic subjects and 46% of ascariasis subjects,¹⁵⁾ suggesting that a potential role of paramyosin as an important cross-reactive allergen in HDMs and AI.

A tropomyosin from *A. lumbricoides*, Asc l 3, has been reported to show cross-reactivity with tropomyosin from Dp¹⁴⁾ and the American cockroach, *Periplaneta Americana*.²¹⁾ IgE-reactive AI, Df, and Dp antigens, which were detected in immunoblot analyses using sera from Bangladeshi children, with molecular weights of 40 (Fig. 1, k), 37, and 36 kDa (data not shown) respectively, can be considered to be tropomyosins (Asc l 3, Der f 10, and Der p 10), although tropomyosin does not appear to be the main cross-reactive antigen in binding to IgG induced in rabbits immunized with AI extract (Figs. 2B and 3C).

Very little or no reactivity to the Dp and cockroach Bg antigens was detected in sandwich ELISA (Figs. 2A and 4) and immunoblot analysis (Figs. 2B and 3C). These are unexpected results, because previous studies reported human IgE cross-reactivity among *Ascaris*, Dp, and cockroach antigens,^{14,21)} and because the amino acid sequences of homologous antigens as between Dp and Df show high amino acid identities (>80%).^{22,23)} Our results suggest that AI antigens have antigenic B-cell epitopes in common with Df, but not Dp (Figs. 2 and 3). However, since amino acid sequences of the HDM paramyosin group 11 allergens from the two species, Der f 11 and Der p 11, are 97% identical,²⁴⁾ it is not plausible to attribute that such a small change in the protein sequence to critically different binding of polyclonal antibodies. Thus the cause of the differing antigenicity between the approximately 100 kDa proteins from Df and Dp (Fig. 3C) remains unknown, but the possibility that unknown post-transcriptional modification of the critical difference in antigenicity cannot be excluded.

Immunization with *Ascaris* antigens with adjuvants other than Freund's complete adjuvant, as used in the present study, and infection with live nematodes may induce anti-*Ascaris* antibodies cross-reactive to both the Df and the Dp antigen. Helminth and HDM themselves produce molecules that have adjuvant activity inducing the Th2 immune response.^{25,26)}

In summary, we found that immunization of naïve animals with *Ascaris* antigens from the human parasite *A. lumbricoides* induced the production of antibodies cross-reactive to HDM antigens, suggesting that exposure to *Ascaris* antigens in *Ascaris*-infected individuals induces antibodies cross-reactive to HDM antigens. The present study is the first step towards the establishment of animal models to study the relationship between *Ascaris* infection and mite-induced allergic diseases. Modification of the sensitization protocol should be addressed in future studies to determine whether and if so how initial sensitization to *Ascaris* antigens affects the subsequent sensitization process to environmental HDM antigens in allergic diseases.

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