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Helminth Infection Alters IgE Responses to Allergens Structurally Related to Parasite Proteins

Helton da Costa Santiago, Flávia L. Ribeiro-Gomes,¹ Sasisekhar Bennuru, and Thomas B. Nutman

Immunological cross-reactivity between environmental allergens and helminth proteins has been demonstrated, although the clinically related implications of this cross-reactivity have not been addressed. To investigate the impact of molecular similarity among allergens and cross-reactive homologous helminth proteins in IgE-based serologic assessment of allergic disorders in a helminth-infected population, we performed ImmunoCAP tests in filarial-infected and noninfected individuals for IgE measurements to allergen extracts that contained proteins with high levels of homology with helminth proteins as well as IgE against representative recombinant allergens with and without helminth homologs. The impact of helminth infection on the levels and function of the IgE to these specific homologous and nonhomologous allergens was corroborated in an animal model. We found that having a tissue-invasive filarial infection increased the serological prevalence of ImmunoCAP-identified IgE directed against house dust mite and cockroach, but not against timothy grass, the latter with few allergens with homologs in helminth infection. IgE ELISA confirmed that filaria-infected individuals had higher IgE prevalences to those recombinant allergens that had homologs in helminths. Mice infected with the helminth *Heligmosomoides polygyrus* displayed increased levels of IgE and positive skin tests to allergens with homologs in the parasite. These results show that cross-reactivity among allergens and helminth proteins can have practical implications, altering serologic approaches to allergen testing and bringing a new perspective to the “hygiene hypothesis.” *The Journal of Immunology*, 2015, 194: 000–000.

The prevalence of allergic diseases has increased worldwide during the past 30–40 y (1). Although the reasons underlying this increase in atopy are unclear, it has been suggested that this increase is largely related to increased standards of personal and community hygiene and lower levels of infections because of the widespread use of antibiotics and vaccines (2), a concept known as the “hygiene hypothesis.” Indeed, it is the collective loss of many infections, particularly those caused by parasitic worms (helminths), that leads to a loss of bystander suppression of allergen-specific responses, thereby allowing for the increased prevalence of allergic diseases (3). This has been inferred from many reports that find a decreased prevalence of atopy or other allergic diatheses in helminth-infected patients when compared with helminth-uninfected controls (4–13). There is, however, some contradictory evidence that suggests that hel-

minth infection may actually drive atopy and promote rhinitis (14, 15), allergic asthma (15–19) and nonallergic bronchospasm (20).

Immunological explanations have been proposed for both the suppression and induction of allergic diseases by helminth infection. For example, chronic helminth infection has been associated with an IL-10-dominated regulatory state that impairs both responses to parasite-specific and bystander Ags (21–23), including those that are vaccine deliverable (24–26). In contrast, helminth parasites acutely induce a strong Th2-like polarization that has been associated with the development of allergic diseases and the production of polyclonal IgE (27, 28). Additionally, parasites encode and secrete proteins that have a high degree of identity (or similarity) with known allergens (29, 30) so that following helminth infection the host develops an IgE response to the parasite that can cross-react with aeroallergens.

The best example of cross-reactivity between an allergen and a helminth protein is parasite tropomyosin (31). It has been demonstrated that tropomyosin of *Ascaris lumbricoides* induces IgE that cross-reacts with tropomyosin of house dust mite (HDM; *Dermatophagoides pteronyssinus* [Der p] 10) (32) or of cockroach (*Blattella germanica* [Bla g] 7) (33). Indeed, IgE to Der p 10 not only cross-reacts with tropomyosin of *Onchocerca volvulus*, but it also induces histamine release by anti-parasite IgE-sensitized basophils (34). However, the list of potentially cross-reactive proteins shared among helminths and allergens can be very extensive, with 40% of 499 molecularly defined allergen families having homologs in helminth parasites genomes (30), and recent work has demonstrated that IgE or IgG cross-reactivity between helminth extracts and HDM extracts can be multiantigenic (35, 36).

Indeed, we have also previously demonstrated that helminth/allergen protein cross-reactivity can occur with molecules less conserved than tropomyosin such as GST (37). Abs to the filarial GST have been shown to cross-react with cockroach GST (Bla g 5), proteins that are only 30% identical but where there is extreme

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Abbreviations used in this article: Bla g, *Blattella germanica*; CI, confidence interval; Der p, *Dermatophagoides pteronyssinus*; Fil⁺, filaria-infected; Fil[−], filaria-uninfected; Fil+A, filaria-infected and atopic; Fil+NA, filaria-infected and nonatopic; GM, geometric mean; HDM, house dust mite; Ni-A, filaria-uninfected and atopic; Ni-NA, filaria-uninfected and nonatopic; OR, odds ratio; Phl p, *Phleum pratense* (timothy grass).

identity of conserved key epitopes (37). This finding suggests that cross-reactivity may be more common than thought previously.

To test whether helminth/allergen cross-reactivity can be generalizable and to better understand the implications of such homology on allergen-specific IgE testing, we performed allergen-specific serologic assessments in two different groups of individuals, that is, those with filarial infections and filaria-uninfected healthy controls. We found that filarial infection was associated with IgE reactivity to allergen extracts that contain proteins homologous to those in helminth parasites. In contrast, when allergen extracts had few potential homologs (e.g., timothy grass extract) with filarial Ags, there was little to no IgE-based cross-reactivity. We could similarly demonstrate the same phenomenon in helminth-infected mice. Our data therefore suggest that helminth infection can modify sensitization to environmental allergens because of protein similarity, a finding that may alter our approach to allergic testing and to the understanding of the hygiene hypothesis.

Materials and Methods

Patients and sera

Sera from well-characterized filaria-infected (Fil⁺) adult individuals were used in this study. All patients were seen at the Clinical Parasitology Section of the Laboratory of Parasitic Diseases under protocols approved by the Institutional Review Board of the National Institute of Allergy and Infectious Diseases and registered (NCT00001230 and NCT00001645). Written informed consent was obtained from all subjects. The diagnosis of a filarial infection was based on well-established and previously described stringent criteria (38). All but four were parasitologically proven (either positive identification of appropriate parasite or parasite DNA in blood, skin snips, or tissue biopsy by microscopy or PCR or positive circulating Ag test for *Wuchereria bancrofti*). The Fil⁺ group in this study was composed of 134 patients with *Loa loa* ($n = 87$), *O. volvulus* ($n = 32$), or *W. bancrofti* ($n = 14$), and one patient was infected with both *L. loa* and *O. volvulus*. Among the 134, 108 were temporary residents of or travelers to filaria-endemic regions, whereas 26 were indigenous to these same regions. Sera from 165 filaria-uninfected (Fil⁻; healthy) individuals were obtained from the Department of Transfusion Medicine, Clinical Center, National Institutes of Health, under protocols approved by the Clinical Center (National Institutes of Health) Institutional Review Board.

All sera were tested for IgE to common allergens using Phadiatop technology (Phadia, Uppsala, Sweden). Phadiatop is a serum-based semiautomated test to detect IgE against a balanced mix of the most prevalent allergens in a given geographic area. The test used for the present study included grasses, trees, weeds, cat, dog, mites, cockroach, and molds. Following the manufacturer's recommendations, serum samples with Phadiatop levels <0.35 kUA/l were considered negative and categorized as nonatopic whereas samples with levels of 0.35 kUA/l or above were considered atopic. Based on these data, the 299 subjects were divided into four groups based on their atopic and filarial infection status: 1) Fil⁻ and nonatopic (Ni-NA), $n = 92$ individuals; 2) Fil⁻ and atopic (Ni-A), $n = 73$; 3) Fil⁺ and nonatopic (Fil+NA), $n = 53$; and 4) Fil⁺ and atopic (Fil+A), $n = 81$. Phadiatop-positive subjects were further tested for IgE directed against HDM (Der p), cockroach (Bla g), and timothy grass (*Phleum pratense* [Phl p]) using Immunocap assays (Phadia).

Recombinant allergens

Recombinant Der p 1, Der p 2, Der p 7, Phl p 2, Phl p 6, Phl p 7, Bla g 6, and Bla g 4 were purchased from Indoor Biotechnologies (Charlottesville, VA). Der p 10 was obtained as described previously (34).

ELISA for IgE and IgG anti-recombinant allergens

Measurements of human allergen-specific IgE, IgG, and IgG4 were performed by ELISA. Flat-bottom plates (Immulon 4; Dynatech Laboratories, Chantilly, VA) were coated overnight at 4°C with 1 µg/ml Ag in PBS followed by washing with PBS and 0.05% Tween 20 (Sigma-Aldrich, St. Louis, MO). Plates were then blocked with PBS/BSA 1% for 1 h at room temperature. Serum samples were diluted in PBS/BSA 1% and incubated overnight at 4°C. Plates were then washed and incubated with polyclonal goat anti-human IgE (R&D Systems, Minneapolis, MN), monoclonal mouse anti-human IgG4 (Hybridoma Reagent Laboratories, Baltimore, MD), or alkaline phosphatase-conjugated goat anti-human IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 h at room tem-

perature. After washing, the plates were incubated with alkaline phosphatase-conjugated anti-goat IgG or anti-mouse IgG for the IgE and IgG4 plates for 1 h at room temperature. Plates were again washed, and *p*-nitrophenylphosphate, disodium salt (Sigma-Aldrich) was added at 1 mg/ml in sodium carbonate buffer (KD Medical, Columbia, MD). Colorimetric development was detected at 405 nm using a microplate reader (Molecular Devices, Sunnyvale, CA), and optical densities were used as a surrogate assessment of the Ab levels. Several dilutions of the samples were tested to give the best signal-to-background ratio, and dilutions selected were 1:50 for IgE and 1:400 for IgG. Geometric mean (GM) + 2 SD of the Ab levels of the Ni-NA group were used to set cut-off values to identify individuals positive and negative for Abs to the different allergens (Supplemental Fig. 1). For responses to Der p allergens a subset of the 109 samples were used: 1) Ni-NA, 23 individuals; 2) Ni-A, 37 individuals; and 3) Fil+A, 49 individuals. The *N* for Phl p recombinant allergens was 99, distributed as follows: 1) Ni-NA, 35 individuals; 2) Ni-A, 30 individuals; and 3) Fil+A, 34 individuals. These numbers were selected to measure all the positive samples in Ni-A and Fil+A groups with enough sera for our analysis and at least 20 Ni-NA sera for cut-off determinations. Because of insufficient serum volume for some samples, Ab measurements of some of the allergen ELISAs have slightly fewer measurements, as can be observed in the Results and in Table III. IgE to recombinant cockroach allergens had been determined previously (37).

For the mouse IgE ELISA, similar procedures were performed with the following modifications: 1) mouse sera were tested at a different dilutions for better signal-to-background ratio, and dilutions of 1:10 were used in all experiments; and 2) polyclonal goat anti-mouse IgE (Abcam, Cambridge, MA) was used as the detection Ab and incubated for 1 h at room temperature followed by washing and incubation with alkaline phosphatase-conjugated anti-goat IgG (Jackson ImmunoResearch Laboratories). The ELISA was developed as described above, and analysis was performed comparing noninfected and infected mouse IgE levels directly.

Mouse infection and skin testing

BALB/c female mice, 6–8 wk old, were purchased from The Jackson Laboratory, housed at an Association for the Assessment and Accreditation of Laboratory Animal Care–approved facility at the National Institute of Allergy and Infectious Diseases, and studied under an animal study proposal approved by the National Institute of Allergy and Infectious Diseases Animal Care and Use Committee (ASP LPD6). Mice were inoculated per os using a gavage tube with 200 infective third-stage larvae of *Heligmosomoides polygyrus*. After 2 wk of infection, animals were treated with pyrantel pamoate (50 mg/kg) and reinfected after 2 wk with 200 third-stage larvae of *H. polygyrus* for an additional 10 d when the animals were skin tested and bled. Mouse ear swelling assays (39) with modifications were performed to evaluate skin sensitivity (37). Mice were injected s.c. with 10 µl PBS containing 10 µg of the indicated allergens in the ear, and thickness was measured with a caliper before and 15, 30, and 60 min after allergen injection.

In silico analysis

Official allergens list from HDM, cockroach, and timothy grass extracts were obtained through World Health Organization/International Union of Immunological Societies (<http://allergen.org/>), and allergen amino acid sequences were obtained at UniProt (<http://uniprot.org/>). Sequences were assessed for homology by searching the National Center for Biotechnology Information Basic Local Alignment Search Tool site (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). We used the Blastp algorithm with blossom62 matrix and conditional compositional score matrix adjustment and an expected value of $<10^{-5}$ cut-off. The *L. loa* genome was used as a representative helminth because an allergen conserved with one helminth is almost always conserved in other helminth genomes at comparative levels of amino acid identity (Ref. 30 and data not shown).

Statistical analysis

GraphPad Prism v5.0 (GraphPad Software, San Diego, CA) was used for all of the statistical analyses (a one-tailed Fisher exact test, odds ratios [OR] with confidence intervals [CI], or Kruskal–Wallis test for human samples, and a Mann–Whitney *U* test or two-way ANOVA for animal data).

Results

We have previously demonstrated that among 499 allergens, there are a considerable number with significant homologs in parasitic nematodes (30). To investigate further the consequences of such levels of homology, we performed serological testing on 134 filaria-infected patients and 165 blood bank donors. The filaria-infected

group had a median age of 38 y (range, 16–92 y) and was 60% male. The group was 76% white, 21% African American, and 4% other. Because the samples of the healthy donors were anonymized, we only know that they came from a subset of donors whose gender distribution was 50% male, with an age range of 18–65 y (median, 46 y) and who were 53% white, 30% African American, and 17% other.

Serum from all the individuals was tested using a Phadiatop assay that utilizes a mix of environmental allergens. We found that Fil⁺ individuals were more likely to be atopic as defined by a positive Phadiatop assay test (Table I): 60% (81 of 134) of the Fil⁺ individuals were positive in these assays compared with 44% (73 of 165) of the Fil[−] subjects ($p = 0.008$). Based on these results, we then divided the entire cohort into four groups: Ni-NA, Ni-A, Fil+NA, and Fil+A. We then measured the levels of polyclonal IgE in these four groups, as helminth infection is known to induce polyclonal IgE. Indeed, Fil⁺ individuals had the highest IgE levels in the plasma (Fig. 1A) and those levels were further increased in the presence of coincident atopy, that is, the Fil+A group. As shown in Fig. 1A, the Fil+A group had a GM IgE level of 702.5 kU/l (CI 491.0–1005), whereas the Fil+NA group had a GM of 157.0 kU/l (CI 110.4–223.2), the Ni-A group had a GM of 44.6 kU/l (CI 34.23–58.22), and the Ni-NA group had a GM of 11.31 kU/l (CI 9.33–13.0).

Sera from both the Ni-A and Fil+A groups were next screened for IgE Abs specific for HDM, cockroach, and timothy grass (Table I). We found an increased likelihood for the Fil+A group to be positive for IgE Abs to HDM (OR 1.95, CI 1.02–3.77) or cockroach (OR 5.92, CI 2.88–12.19), but not to timothy grass (OR 1.11, CI 0.59–2.09). Interestingly, whereas the increased prevalence of IgE to common allergen extracts could be observed for HDM and cockroach (Table I), the magnitude of the levels of allergen-specific IgE was comparable between the two groups (Fig. 1B), that is, the Ni-A and Fil+A groups had similar levels of IgE anti-HDM (2.34 versus 2.41, respectively, $p > 0.05$), cockroach (2.16 versus 2.07, $p > 0.05$), and timothy grass (3.27 versus 2.54, $p > 0.05$).

To gain insight into the level of homology among HDM, cockroach, and timothy grass allergen extracts and filarial parasites, we performed in silico analysis using amino acid sequences of allergens present in these extracts and compared them to the putative proteome of *Brugia malayi*, a representative filarial worm. We found that from the allergens of HDM listed in the list of World Health Organization/International Union of Immunological Societies, 70% (12 of 17) of HDM allergens and 55% (5 of 9) of the cockroach allergens had proteins homologous with *B. malayi*, including the major allergens of both extracts: Der p 1, Der p 2, Der p 11, Der p 23, Bla g 2, Bla g 5, and Bla g 6 (Table II). In contrast, only two of nine (22%) allergens from timothy grass extract showed homologs in *B. malayi* and both (Phl p 7 and Phl p 12) are considered to be minor allergens.

Having previously demonstrated that cross-reactivity of Bla g 5 to helminth GST could explain the increased levels of IgE to cockroach observed in helminth-infected individuals (37), we

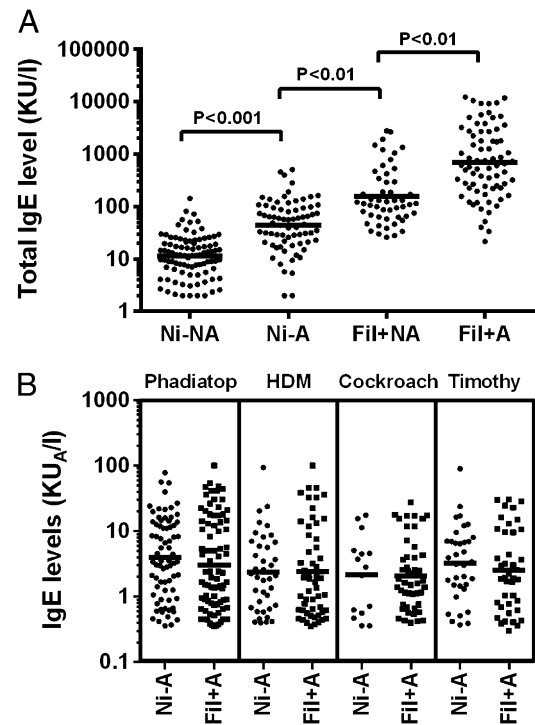


FIGURE 1. Filarial infection induces high levels of IgE that is potentiated by atopy in infected individuals. ImmunoCAP technology was used to assess total (polyclonal) IgE (A) or IgE directed to specific allergen extract in Phadiatop-positive individuals depicted in Table I (B). Sera are from blood bank donors (noninfected) or filaria-infected patients. Individuals were classified as atopic or nonatopic. Each dot represents one individual, and the horizontal lines represent the GM. Statistics were performed using a Kruskal–Wallis test.

asked whether a similar mechanism was at play for HDM. Thus, to investigate whether cross-reactivity was likely to be responsible for the increased prevalence of IgE to HDM but not to timothy grass extract in helminth infections, we developed ELISAs to assess Ag-specific IgE to the recombinant allergens with (Der p 1, Der p 2, and Phl p 7) and without (Der p 7, Phl p 2, and Phl p 6) known homologs in the filarial proteome. We assayed IgE from Fil+A and Ni-A groups using the Ni-NA group as a reference to set the cut-off values for the ELISAs (Supplemental Fig. 1). Despite the fact that helminth infection increased some specific background IgE levels (Supplemental Fig. 1), possibly associated with dramatic increase in total IgE (27), such increases had little impact on the prevalence analyses, as we could still observe a clear effect of helminth infection on both IgE and IgG (not associated with helminth-induced increases in total IgG) levels against allergens bearing helminth homologs (Table III) but not against allergens without parasite homologs. For example, the prevalence of IgE to Der p 1 increased from 32.4% (12 of 37) in the Ni-A group to 71.4% (35 of 49) in the Fil+A group ($p < 0.001$, OR 5.2, CI 2.06–13.15), and IgG prevalence increased from

Table I. Filaria-infected individuals display increased prevalence of allergen-specific IgE

Allergy Test ^a	Fil [−] , % (no.)	Fil ⁺ , % (no.)	OR (95% CI)	<i>p</i> Value
Phadiatop	44.2 (73/165)	60.4 (81/134)	1.926 (1.21–3.06)	0.008
HDM	53.4 (39/73)	69.1 (56/81)	1.953 (1.01–3.77)	0.0486
Cockroach	20.5 (15/73)	60.4 (49/81)	5.921 (2.87–12.1)	<0.0001
Timothy grass	47.9 (37/73)	50.6 (41/81)	1.113 (0.59–2.09)	0.7498

^aOnly samples positive for Phadiatop were used for HDM, cockroach, and timothy grass ImmunoCAP assays.

Table II. HDM and cockroach allergens have homologs in filarial parasites using in silico analysis

	Allergen ^a	Accession No. ^b	Biological Function	Prevalence of IgE (%) ^c	<i>L. loa</i> Homolog	Accession No. ^b	Identity (%)	E-Value
HDM	Der p 1	B5AYU7	Cysteine protease	85–100	L1 cysteine protease	E1G9M8	33	e-30
	Der p 2	P49278	NPC2	63–97	L1 ML protein	E1FJ34	23	e-05
	Der p 3	P39675	Serine protease	9–97	L1 uncharacterized protein	E1GBY4	39	e-19
	Der p 4	Q9Y197	α-Amylase	6–45				
	Der p 5	P14004		23–90				
	Der p 6	P49277	Chymotrypsin	41–65				
	Der p 7	P49273	Protease	17–53				
	Der p 8	P46419	GST	9–75	L1 uncharacterized protein	E1FK96	33	e-23
	Der p 9	Q7Z163	Serine protease	92	L1 uncharacterized protein		31	e-09
	Der p 10	O18416	Tropomyosin	6–32	L1 tropomyosin	J0DYI5	73	e-128
	Der p 11	Q6Y2F9	Paramyosin	50–75	L1 paramyosin	E1FX82	51	0
	Der p 14	Q8N0N0	Vitellogenin	0–10				
	Der p15	Q4JK69	Chitinase	8–70	L1 endochitinase	E1GIC3	34	e-61
	Der p 18	Q4JK71	Chitinase	63	L1 endochitinase		25	e-33
	Der p 20	B2ZSY4	Arginine kinase	7–44	L1 arginine kinase	J9EDX6	64	e-172
	Der p 21	Q2L7C5	Unknown	18–56				
	Der p 23	L7N6F8	Chitin binding protein	74	L1 uncharacterized protein	J0M5S6	45	e-05
Cockroach	Bla g 1	Q9UAM5		8–77				
	Bla g 2	P54958	Aspartic protease	35–57	L1 aspartyl protease 6	E1GIM4	29	e-16
	Bla g 3	D0VNY7	Hemocyanin	Not known				
	Blag g 4	P54962	Lipocalin	17–37				
	Bla g 5	O18598	GST	37–70	L1 uncharacterized protein	E1FK96	27	e-17
	Bla g 6	Q1A7B3	Troponin C	50	L1 troponin C	E1GB15	52	e-53
	Bla g 7	Q9NG56	Tropomyosin	17	L1 tropomyosin	J0DYI5	70	e-115
	Bla g 8	A0ERA8	Myosin L chain	Not known	Miosin regular L chain	E1FQY9	41	e-27
	Bla g 11	Q2L7A6	α-Amylase	41				
	Phl p 1	Q40967	β-expansin-like	68–97				
Timothy grass	Phl p 2	P43214	Expansin-like	56–89				
	Phl p 4	Q2I6V7	Berberine bridge enzyme	55–92				
	Phl p 5	Q9AT26	RNase	50–100				
	Phl p 6	O65869	P particle-associated protein	52–75				
	Phl p 7	O82040	Polcalcin	5–30	L1 uncharacterized protein	J0DZZ5	43	e-09
	Phl p 11	Q8H6L7	Soybean trypsin-like protease	32–42				
	Phl p 12	P35079	Profilin	10–24	L1 profiling	E1FUW5	37	e-19
	Phl p 13	Q9XG86	Polygalacturonase	50–56				

Results show a Blastp search using conditional compositional score matrix adjustment on blossom62.

^aNomenclature of allergen (World Health Organization/International Union of Immunological Societies).

^bAccession no. from UniProtKB/TrEMBL database.

^cAggregated literature data are found on <http://www.allergome.org>.

E-Value, expected value.

8.1% (3 of 37) in the Ni-A group to 33.3% (16 of 48) in the Fil+A group ($p = 0.008$, OR 5.6, CI 1.50–21.3). For Der p 2, the IgE prevalence increased from 27% (10 of 37) in the Ni-A group to 49% (24 of 49) in the Fil+A group ($p = 0.047$, OR 2.6, CI 1.03–6.48) and IgG prevalence increased from 5.4% (2 of 37) to 25.0% (12 of 48), respectively ($p = 0.018$, OR 5.8, CI 1.21–27.9). IgE anti-Phl p 7 allergen was incremented from 6.7% (2 of 30) in the Ni-A group to 29% (9 of 31) in the Fil+A group ($p = 0.024$, R 5.7, CI 1.12–29.2) and with an increase in IgG from 6.7% (2 of 30) to 32.3% (10 of 31), respectively ($p = 0.021$, OR 6.6, CI 1.32–33.7). For allergens without homologs, that is, Der p 7, Phl p 2, and Phl p 6, despite a small trend toward increased IgE levels postinfection, only Der p 7 showed a significant increase in prevalence from 10.8% (4 of 37) in the Ni-A group to 31% (14 of 45) in the Fil+A group ($p = 0.033$, OR 3.7, CI 1.1–12.5). Importantly, none showed significant increases in IgG prevalences (Table III). IgG4 was also assessed, and, despite increased levels of IgG4 against crude parasite extract (BMA)

in infected individuals, few patients showed positive IgG4 against the recombinant allergens with no differences seen between the Ni-A and Fil-A groups (data not shown). These data suggest that the infection was not sufficiently longstanding to induce IgG4 to cross-reactive homologs (40), in accordance to our infected population, which was composed mostly of individuals with relatively acute infections.

In humans, it is impossible to dissect the Th2 adjuvant effect of helminth infection from the effects of cross-reactivity on IgE levels, because during infection individuals might also be exposed to environmental allergens. To evaluate the effects of helminth infections on the IgE levels to several allergens with and without homologs in a more controlled manner, we used BALB/c mice experimentally infected with *H. polygyrus*, an intestinal nematode that also induces strong Th2 immune response with marked IgE production. We clearly could observe that *H. polygyrus* infection induced IgE to allergens with (Fig. 2A) and without (Fig. 2B) parasite homologs, but those allergens with homologs (i.e., Der

Table III. Filarial infection increases IgE and IgG Abs to specific allergens (with helminth homologs) among atopic individuals

Allergen	Homolog in Helminth Parasites	IgE ^a				IgG			
		Prevalence %		Odds (CI)	<i>p</i> Value ^b	Prevalence %		Odds (CI)	<i>p</i> Value ^b
		Ni-A	Fil-A			Ni-A	Fil-A		
Der p 1	Cathepsin ML protein	<i>n</i> = 37	<i>n</i> = 49			<i>n</i> = 37	<i>n</i> = 48		
Der p 2		32.4	71.4	5.2 (2.06–13.15)	0.0004	8.1	33.3	5.6 (1.50–21.3)	0.008
Der p 7		27.0	49.0	2.6 (1.03–6.48)	0.047	5.4	25.0	5.8 (1.21–27.9)	0.018
		10.8	31.0	3.7 (1.1–12.5)	0.033	8.1	19.1	2.8 (0.70–11.3)	0.209
Phl p 2	EF hand family	<i>n</i> = 30	<i>n</i> = 34			<i>n</i> = 30	<i>n</i> = 34		
Phl p 6		33.3	55.9	2.5 (0.91–7.0)	0.083	33.3	41.1	1.4 (0.50–3.88)	0.608
Phl p 7		30.0	52.9	2.3 (0.83–6.54)	0.079	16.7	29.4	2.0 (0.62–6.99)	0.255
		6.7	29.0	5.7 (1.12–29.2)	0.042	6.7	32.3	6.6 (1.32–33.7)	0.021

^aIgE and IgG anti-recombinant allergens evaluated by ELISA as described in *Materials and Methods*.
^bStatistical evaluation was by a Fisher exact test.

p 1, Der p 2, Der p 10, Phl p 7, Bla g 5, and Bla g 6) were more likely to be associated with the presence allergen-specific IgE than those without homologs (i.e., Der p 7, Phl p 2, and Bla g 4). Among the allergens without homologs in the parasite, only Der p 7 showed significant increases of IgE levels with *H. polygyrus* infection (*p* = 0.006). On average, the *H. polygyrus* infection induced an increase in 20% of specific IgE levels for allergens without homologs in helminthes, whereas the increase of IgE was at least twice that (40%) for allergens with homologs in the parasite (*p* = 0.01).

We have previously demonstrated that cross-reactive IgE can induce cross-reactive allergic reactivity in animal models (37). To

test whether this finding was generalizable, we tested representative allergens from HDM and timothy grass in BALB/c mice infected twice with *H. polygyrus* (Fig. 3). We found that Der p 1 (an HDM allergen with homolog), but not Der p 7 (allergen without homolog), induced an immediate hypersensitivity reaction in the skin of infected mice (Fig. 3). Similarly, Phl p 7 (a timothy grass allergen with a helminth homolog), but not Phl p 2, induced an immediate hypersensitivity reaction in the ears of mice (Fig. 3).

Discussion

Infection with helminth parasites can induce a state of immune regulation that modulates allergic and autoimmune-mediated inflammatory diseases. Such anti-inflammatory states are thought to be driven by IL-10 (4, 23), T and B regulatory cells (41, 42), non-IgE allergen-specific Abs, such as IgG1 and especially IgG4 (43–45), and by parasite-specific products with the ability to promote immune modulation (46). Helminth infection has therefore been used in clinical trials to reestablish the immune balance in individuals with chronic inflammatory disease such as asthma (47, 48), inflammatory bowel disease, and other autoimmune diseases (49).

The effects of helminth infection on allergy have been widely investigated with widely varying conclusions. Although attention has been given to the modulatory effects of helminth infections on the clinical manifestations of allergy, there are data that suggest that helminth infection can increase allergic symptoms. Additionally, clinical trials using experimental infections with helminths in humans to treat allergic diseases have failed to show promise in inducing allergic symptom relief (47, 48) or in the modulation of the immune response to aeroallergens (50). In fact, the many aspects of the interface between allergy and helminths interaction suggest that 1) helminth infections can promote a Th2 balance favoring IgE class switch or polyclonal B cell activation with massive IgE production, and 2) IgE raised against helminth can cross-react with allergens favoring cross-sensitization. Our data and those of others suggest that both processes occur in helminth infections (34, 37, 51).

In the present study, we suggest that cross-reactivity among allergens and helminth proteins can be very common and may alter the serology-based diagnostics often used for allergic diseases in parasite-endemic countries. There is a great effort toward more reliable, objective, and simple diagnostic tools for allergic diseases (52), but progress has been limited. Serological tests to screen for allergy have been developed and improved to bring high quality, robust, safe, and reliable serologic tools. We used ImmunoCAP technology to assess the allergic status on filaria-infected and -uninfected subjects and found that parasite infection status had a significant impact on the prevalence of positive allergen-specific IgE. Additionally, we were able to replicate these findings in an experi-

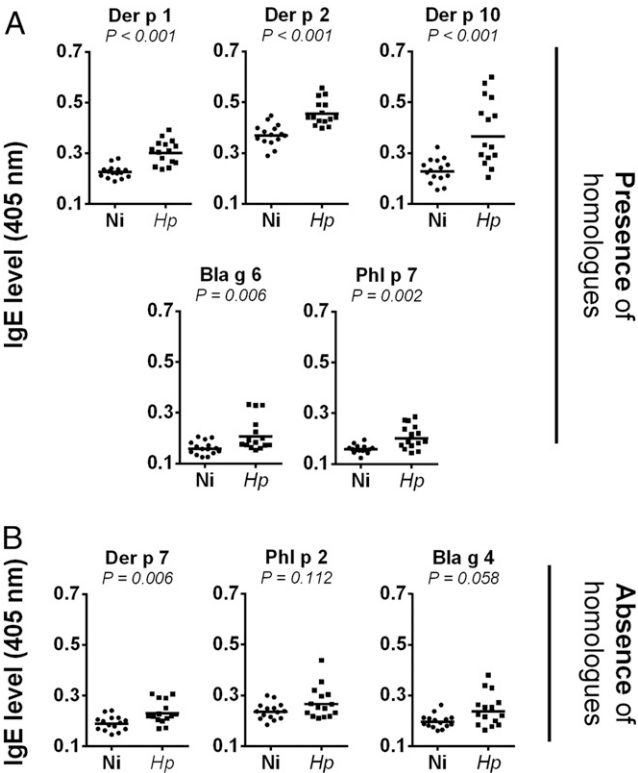


FIGURE 2. Experimental helminth infection can influence allergen-specific IgE. Sera from naive BALB/c mice (Ni) or animals infected twice with the murine helminth *H. polygyrus* (Hp) and bled 10 d after second infection were used in an ELISA assay for allergen-specific IgE using recombinant allergens from HDM (Der p), cockroach (Bla g), and timothy grass (Phl p) displaying helminth homologs (A) or not (B). Each dot represents one animal pooled from three independent experiments (*n* = 15). Lines represent GM. The *p* values were calculated by a Mann–Whitney *U* test.

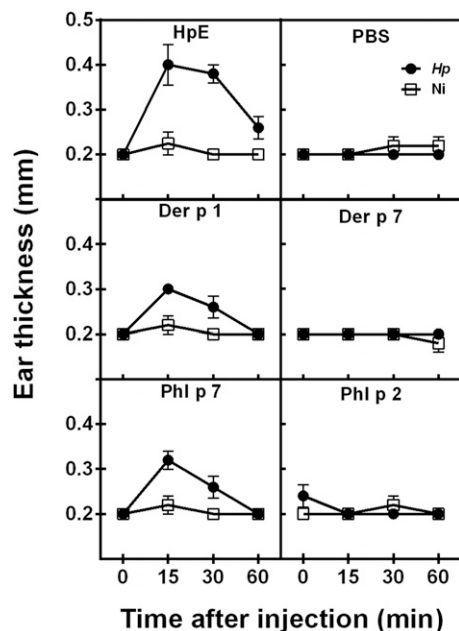


FIGURE 3. Experimental helminth infection can induce cross-sensitization to allergens using mouse skin tests. BALB/c animals infected twice with *H. polygyrus* were skin tested 10 d after the second infection for allergens with and without homologs in parasites. Ears were injected with 10 μ g recombinant allergen and ear thickness was measured at the indicated times with a caliper. Symbols represent means $\pm$ SE of five ears per group. *H. polygyrus* and Ni groups differed in their responses to parasite extract (HpE), Der p 1, and Phl p 7 ($p < 0.05$ by two-way ANOVA). Experiments were performed twice with similar results.

mental model using an intestinal nematode with a totally distinct life cycle, suggesting that this finding is not restricted to filarial nematodes. This particular observation, that helminth infection drives allergen-specific IgE, had been observed previously for *A. lumbricoides* (15, 20) but has drawn little attention so far.

Our results help to understand why infections with helminths may be associated with increased IgE levels to common allergens, even when dissociated from allergic symptoms. We found that filarial infections in humans and *H. polygyrus* infection in mice induced IgE to allergens with homologs in helminth parasites. Interestingly, we could observe in our immunoassays a statistically insignificant moderate increase in IgE, and less markedly in IgG, to allergens for which there was no homolog in helminths, an effect that we think is associated with the induction of polyclonal IgE by worm infections (27, 28) or even to a Th2 adjuvant effect of helminth infection. Indeed, filarial infection was associated with an increased level of IgE from 5- (Fil+NA) to 25-fold (Fil+A) above the levels observed in the Ni-A group. Although this strong polyclonal response could be associated with small increases in background IgE and IgG levels to environmental allergens, these effects could be easily distinguished from the stronger effect mediated by molecular homology. This can be clearly observed on IgE levels and, even more clearly, on IgG levels to recombinant allergens.

A limitation of our study may be that the cut-offs used in our analysis (GM of the Ni-NA group plus 2 SDs) may have underestimated the prevalence of allergen-specific IgE prevalence, a cut-off that was kept throughout our analyses allowing comparison between groups. Despite this limitation, all of the results generated in the present study, including the ImmunoCAP and mouse data, are consistent with each other.

Using IgE measurements to allergen extracts, in which the major allergens shared homologs in the parasite (such as HDM and

cockroach), parasite-infected patients had higher prevalences of measurable allergen-specific IgE. If increased prevalence in IgE to HDM and cockroach was only due to polyclonal IgE production or to the Th2 adjuvant effect of the helminth infection, there would be no distinction of IgE levels between HDM and cockroach with timothy grass extract (an extract that lacks important homologs with helminths), which showed no increase in allergen-specific IgE prevalence in helminth infection. Furthermore, the data cannot support that one group had exposure to less hygienic conditions than the others given that even within an allergen extract there was preferential induction of IgE by helminth infection to Phl p 7 but not other Phl p allergens, or of IgG to anti-Der p 1 but not Der p 7. Additionally, our previous results showed that helminth infection induces cross-reactive Abs to Bla g 5 (cockroach GST, an allergen with helminth homologs), but not to Bla g 4 (another cockroach allergen without helminth homolog). Therefore, these results suggest that the presence of Ab to parasite Ags can alter dramatically the serological response to environmental allergens. These results thus suggest that presence of parasite homologs can alter dramatically serological allergy tests. The tendency of the immune system to react preferentially to epitopes showing some level of similarity to one seen before is a common concept in virology known as "original antigenic sin" (53), which suggests that a subsequent viral infection can have a different outcome depending on whether the host had been in contact with a similar virus previously. Our data suggest that a similar mechanism may be operating in helminth infection, but future (and longitudinal) studies will need to examine this possibility carefully.

Although we have not performed detailed experiments to investigate immunologic cross-reactivity in the present study, Ab directed to helminth proteins cross-reacts with highly conserved environmental allergens homologs such as tropomyosins (32–34) and with some less well-conserved proteins such as GST (37). These findings suggest that cross-reactivity may have a broader impact on atopic disorders, as close to 40% of defined allergens have helminth homologs (30). The present result underscores this inference because we found a strong association between helminth infection and the development of allergen-specific IgE. Whether this is merely related to chronicity of infection or other factors remains to be seen. Additionally, the clinical implications of these findings are yet to be determined because allergen-specific IgE sometimes will not be associated with clinical symptoms, especially in helminth-infected individuals (15, 20).

Another intriguing finding was the increase in IgG levels against allergens with parasite homologs. For example, whereas IgG levels against allergens displaying parasite homologs increased in the presence of helminth infection, none of the allergens without homologs showed such increases. Allergen-specific IgG have been shown to have regulatory effects against allergy (44, 45, 54) and may be an additional immunological mechanism to counterregulate the proallergic effects of IgE.

We think that the implications of helminth infection-associated anti-allergen IgE can go beyond the serologic-based assays used in allergic diseases. Our animal data suggest that this increase in prevalence of allergen-specific IgE and sometimes allergies associated with helminth infections may be a consequence of the parasite-specific IgE generated during infection. Although very frequently helminth infections are associated with modulation of allergic responses, cross-reactive IgE can bind to environmental allergens and change the balance of regulatory/proallergic effects of these parasites, suggesting a novel mechanism to explain how atopy and allergic disorders can be induced by helminth infection.

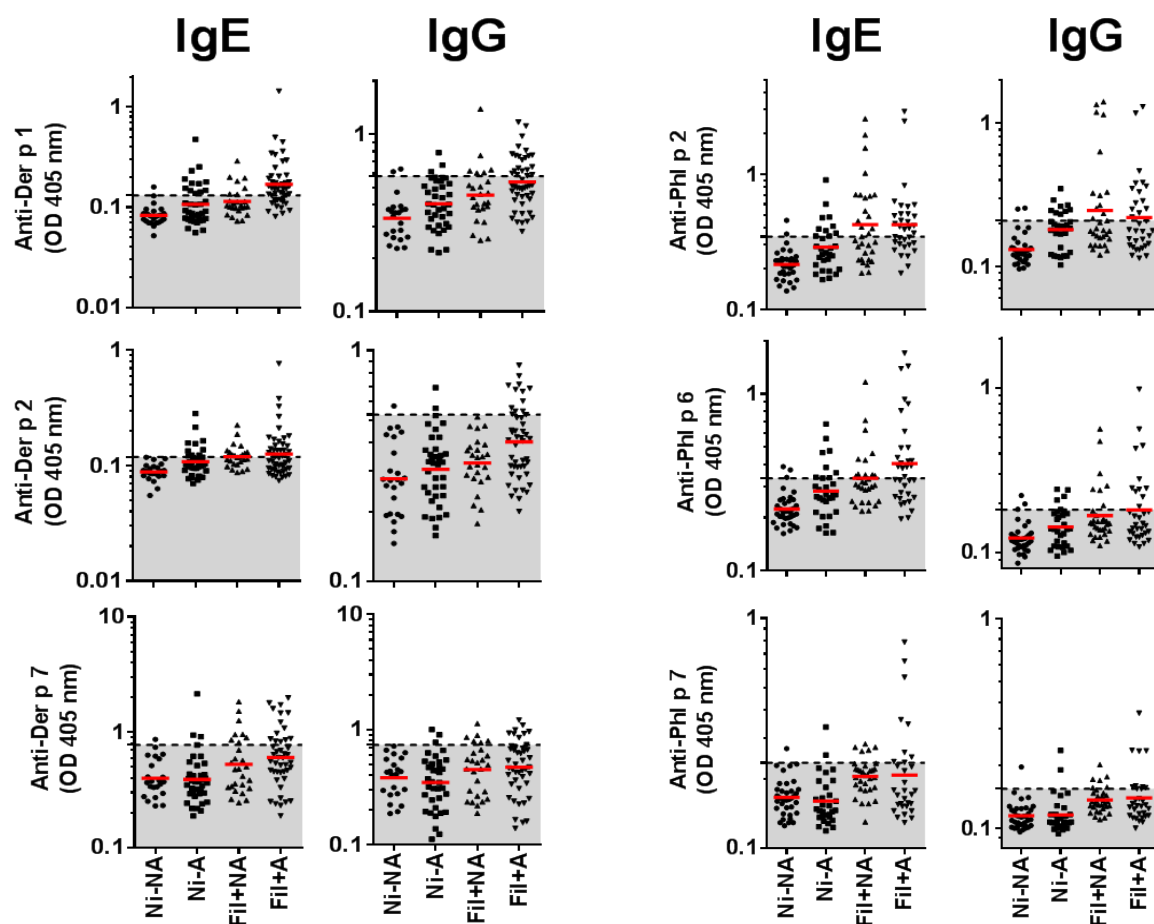
Disclosures

The authors have no financial conflicts of interest.

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IgE and IgG levels of anti-Der p and Phl p allergens in serum of different volunteers groups. Sera were diluted 1:50 (IgE) or 1:400 (IgG) and used to perform ELISA as described in Methods. Geometric mean (GM) + 2 SD of the Ab levels of the Ni-NA group were used to set cut off values (dotted lines) to identify individuals positive (above gray area) and negative (under gray area) for Abs to the different allergens. Red lines represent GM. Each dot is an individual.