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*J Immunol* 2015; 194:93-100; Prepublished online 17 November 2014;
doi: 10.4049/jimmunol.1401638
http://www.jimmunol.org/content/194/1/93

Supplementary Material
http://www.jimmunol.org/content/suppl/2014/11/15/jimmunol.1401638.DCSupplemental

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

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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: 93–100.

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 helminth 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 tropomysin (31). It has been demonstrated that tropomysin of *Ascaris lumbricoides* induces IgE that cross-reacts with tropomysin of house dust mite (HDM; *Dermatophagoides pteronymissinus* [Der p] 10) (32) or of cockroach (*Blattella germanica* [Bla g] 7) (33). Indeed, IgE to Der p 10 not only cross-reacts with tropomysin 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 tropomysin 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...
identity of conserved key epitopes (37). This finding suggests that
cross-reactivity may be more common than thought previously.

To test whether helmint/allergen cross-reactivity can be
generalizable and to better understand the implications of such ho-
metry on allergen-specific IgE testing, we performed allergen-
specific serologic assessments in two different groups of individ-
uals, 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 ho-
monologous to those in helmint 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 helmint-
infected mice. Our data therefore suggest that helmint infection
may 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 NCT0001645).
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 (Blg b), and timothy grass (Phleum pratense
[Phl p]) using Immunocap assays (Phadia).

Recombinant allergens

Recombinant Der p 1, Der p 2, Der p 7, Fil p 2, Fil p 6, Fil p 7, Blg b 6, and
Blg b 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
cycloheximide and methyl-xanthine.

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 from 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 bloom62 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
helmint because an allergen conserved with one helmint is almost al-
ways conserved in other helmint 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]
given for conditional ratio, and 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
hematodes (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 34.2–58.22), 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 extracts and filarial parasites, we performed in silico analysis using amino acid sequences of the cockroach allergens had proteins homologous with B. 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, 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 assess Ag-specific IgE to the recombinant allergens with (Der p 1, Der p 2, and Phil p 7) and without (Der p 7, Phil p 2, and Phil 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 helmint 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 helmint infection on both IgE and IgG (not associated with helmint-induced increases in total IgG) levels against allergens bearing helmint 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.

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

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

FIGURE 1. Filarial infection induces high levels of IgE that is potentiated by atopy in infected individuals. ImmunoCAP technology was used to assess total (polycional) 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.
Table II. HDM and cockroach allergens have homologs in filarial parasites using in silico analysis

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Accession No.</th>
<th>Biological Function</th>
<th>Prevalence of IgE (%)</th>
<th>L. loa Homolog</th>
<th>Accession No.</th>
<th>Identity (%)</th>
<th>E-Value</th>
</tr>
</thead>
<tbody>
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<td>HDM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Der p 1</td>
<td>B5AYU7</td>
<td>Cysteine protease</td>
<td>85–100</td>
<td>Li cysteine protease</td>
<td>E1G9M8</td>
<td>33</td>
<td>e-30</td>
</tr>
<tr>
<td>Der p 2</td>
<td>F49278</td>
<td>NPC2</td>
<td>63–97</td>
<td>Li ML protein</td>
<td>E1FJ34</td>
<td>23</td>
<td>e-05</td>
</tr>
<tr>
<td>Der p 3</td>
<td>F39675</td>
<td>Serine protease</td>
<td>9–97</td>
<td>Li uncharacterized</td>
<td>E1GBY4</td>
<td>39</td>
<td>e-19</td>
</tr>
<tr>
<td>Der p 4</td>
<td>Q9Y197</td>
<td>α-Amylase</td>
<td>6–45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Der p 5</td>
<td>P14004</td>
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<td>23–90</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Der p 6</td>
<td>P49277</td>
<td>Chymotrypsin</td>
<td>41–65</td>
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<tr>
<td>Der p 7</td>
<td>P49273</td>
<td>Protease</td>
<td>17–53</td>
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<tr>
<td>Der p 8</td>
<td>P46419</td>
<td>GST</td>
<td>9–75</td>
<td>Li uncharacterized</td>
<td>E1FK96</td>
<td>33</td>
<td>e-23</td>
</tr>
<tr>
<td>Der p 9</td>
<td>Q7Z163</td>
<td>Serine protease</td>
<td>92</td>
<td>Li uncharacterized</td>
<td>E1FJ34</td>
<td>31</td>
<td>e-09</td>
</tr>
<tr>
<td>Der p 10</td>
<td>O18416</td>
<td>Tropomyosin</td>
<td>6–32</td>
<td>Li tropomyosin</td>
<td>J0DY15</td>
<td>73</td>
<td>e-128</td>
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<tr>
<td>Der p 11</td>
<td>Q6Y2P9</td>
<td>Pararosynin</td>
<td>50–75</td>
<td>Li pararosynin</td>
<td>E1FX82</td>
<td>51</td>
<td>0</td>
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<td>Der p 14</td>
<td>Q8N10N0</td>
<td>Viellegenin</td>
<td>0–10</td>
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<td>Der p 15</td>
<td>Q4IK99</td>
<td>Chitinase</td>
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<td>Li endochitinase</td>
<td>E1GIC3</td>
<td>34</td>
<td>e-61</td>
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<td>Der p 18</td>
<td>Q4IK71</td>
<td>Chitinase</td>
<td>63</td>
<td>Li endochitinase</td>
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<tr>
<td>Der p 20</td>
<td>B2ZSY4</td>
<td>Arginine kinase</td>
<td>7–44</td>
<td>Li arginine kinase</td>
<td>J0EDX6</td>
<td>64</td>
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<td>Der p 21</td>
<td>Q2L7C5</td>
<td>Unknown</td>
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<td>Der p 23</td>
<td>L7N6F8</td>
<td>Chitin binding protein</td>
<td>74</td>
<td>Li uncharacterized</td>
<td>J0M5S6</td>
<td>45</td>
<td>e-05</td>
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<tr>
<td>Cockroach</td>
<td></td>
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<tr>
<td>Bla g 1</td>
<td>Q9UAM5</td>
<td>Aspartic protease</td>
<td>8–77</td>
<td>Li aspartyl protease 6</td>
<td>E1GIM4</td>
<td>29</td>
<td>e-16</td>
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<tr>
<td>Bla g 2</td>
<td>P54058</td>
<td>Hemocyanin</td>
<td>35–57</td>
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<tr>
<td>Bla g 3</td>
<td>D10YN7</td>
<td>Lipocalin</td>
<td>17–37</td>
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<td>Bla g 4</td>
<td>P48496</td>
<td>GST</td>
<td>37–70</td>
<td>Li uncharacterized</td>
<td>E1FK96</td>
<td>27</td>
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<td>Bla g 5</td>
<td>P18S98</td>
<td>Tropinon C</td>
<td>50</td>
<td>Li tropinon C</td>
<td>E1GB15</td>
<td>52</td>
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<td>Bla g 6</td>
<td>Q1A7B3</td>
<td>Tropomyosin</td>
<td>17</td>
<td>Li tropomyosin</td>
<td>J0DY15</td>
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<td>e-115</td>
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<tr>
<td>Bla g 7</td>
<td>Q9N5G6</td>
<td>Tropomyosin</td>
<td>17</td>
<td>Li tropomyosin</td>
<td>E1FQ9Y9</td>
<td>41</td>
<td>e-27</td>
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<tr>
<td>Bla g 8</td>
<td>A0EERA8</td>
<td>Myosin L chain</td>
<td>Not known</td>
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<td>Timothy</td>
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<tr>
<td>grass</td>
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<td>Bla g 11</td>
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<td>41</td>
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<td>Q40961</td>
<td>β-expansin-like</td>
<td>68–97</td>
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<td>Phil p 2</td>
<td>P43214</td>
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<td>Q3J67V</td>
<td>Berberine bridge</td>
<td>55–92</td>
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<tr>
<td>Phil p 5</td>
<td>Q9AT26</td>
<td>RNase</td>
<td>50–100</td>
<td>Li uncharacterized</td>
<td>J0DZ335</td>
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<td>Phil p 7</td>
<td>O65869</td>
<td>P particle-associated protein</td>
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<td>Phil p 11</td>
<td>Q6H6L7</td>
<td>Soybean trypsin-like protease</td>
<td>32–42</td>
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<td>P35079</td>
<td>Polifrin</td>
<td>10–24</td>
<td>Li profiling</td>
<td>E1FUW5</td>
<td>37</td>
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<td>Polygalacturonase</td>
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<td></td>
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Results show a Blastp search using conditional compositional score matrix adjustment on blossum62.

1Nomenclature of allergen (World Health Organization/International Union of Immunological Societies).

2Accession no. from UniProtKB/TrEMBL database.

3Aggregated 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-Phil 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 29% (9 of 31) in the Fil+A group (p = 0.008, OR 5.6, CI 1.50–21.3). 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) parasitic homologs, but those allergens with homologs (i.e., Der
The pooled from three independent experiments (\(n=3 0\)) displaying helminth homologs (allergens from HDM (Der p), cockroach (Bla g), and timothy grass (Phl p) were used in an ELISA assay for allergen-specific IgE using recombinant \(H.\) polygyrus the murine helminth.

**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 (\(n=1 5\)). Lines represent GM. The \(p\) values were calculated by a Mann–Whitney \(U\) test.

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).

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<table>
<thead>
<tr>
<th>Allergen</th>
<th>Homolog in Helminth Parasites</th>
<th>IgE*</th>
<th>Odds (CI)</th>
<th>(p) Value*</th>
<th>IgG</th>
<th>Odds (CI)</th>
<th>(p) Value*</th>
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<tbody>
<tr>
<td>Ni-A</td>
<td>Fil-A</td>
<td></td>
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<tr>
<td>Der p 1</td>
<td>Cathepsin</td>
<td>32.4</td>
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<td>ML protein</td>
<td>27.0</td>
<td>49.0</td>
<td>2.6 (1.03–6.48)</td>
<td>0.047</td>
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<td>Der p 7</td>
<td></td>
<td>10.8</td>
<td>31.0</td>
<td>3.7 (1.1–12.5)</td>
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<td>33.3</td>
<td>55.9</td>
<td>2.5 (0.91–7.0)</td>
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<td>Phi p 6</td>
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<td>30.0</td>
<td>52.9</td>
<td>2.3 (0.83–6.54)</td>
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<td>16.7</td>
<td>29.4</td>
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<tr>
<td>Phi p 7</td>
<td>EF hand family</td>
<td>6.7</td>
<td>29.0</td>
<td>5.7 (1.12–29.2)</td>
<td>0.042</td>
<td>6.7</td>
<td>32.3</td>
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*IgE and IgG anti–recombinant allergens evaluated by ELISA as described in Materials and Methods.

*Statistical evaluation was by a Fisher exact test.

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-
even when dissociated from allergic symptoms. We found that may be associated with increased IgE levels to common allergens, (15, 20) but has drawn little attention so far.

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 μg recombinant allergen and ear thickness was measured at the indicated times with a caliper. Symbols represent means ± SE of five ears per group. *H. polygyrus* and Ni groups differed in their responses to parasite extract (HpE), Der p 1, and Phil 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 cycle, suggesting that this finding is not restricted to filarial nematodes, allows us to extend our previous findings to other helminth species with different life cycles. Experimental helminth infection can induce cross-reactive Abs to allergens with and without homologs in parasites. Ears were injected with 10 μg recombinant allergen and ear thickness was measured at the indicated times with a caliper. Symbols represent means ± SE of five ears per group. *H. polygyrus* and Ni groups differed in their responses to parasite extract (HpE), Der p 1, and Phil p 7 (p < 0.05 by two-way ANOVA). Experiments were performed twice with similar results.

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 are differentially directed to helminth proteins cross-react 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 it occurs frequently in helminth infections. Furthermore, the data 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, Abs directed to helminth proteins cross-react 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 it occurs frequently in helminth infections. Furthermore, the data 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.

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.


Supplemental Figure 1: 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.