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Molecular mimicry between cockroach and helminth glutathione S-transferases promotes crossreactivity and cross-sensitization

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Abstract

Background—The extensive similarities between helminth proteins and allergens are thought to contribute to helminth-driven allergic sensitization.

Objective—To investigate the cross-reactivity between a major glutathione-S transferase (GST) allergen of cockroach (Bla g 5) and the GST of *Wuchereria bancrofti* (WbGST), a major lymphatic filarial pathogen of humans.

Methods—We compared the molecular and structural similarities between Bla g 5 and WbGST by in silico analysis and by linear epitope mapping. Levels of IgE, IgG and IgG4 antibodies were measured in filarial-infected and –uninfected patients. Mice were infected with *Heligmosomoides bakeri* (Hb) and skin tested for cross-reactive allergic responses.

Results—These two proteins are 30% identical at the amino acid level with remarkable similarity in the N-terminal region and overall structural conservation based on predicted three-dimensional models. Filarial infection was associated with IgE, IgG, and IgG4 anti-Bla g 5 Ab production, with a significant correlation between Abs (irrespective of isotype) to Bla g 5 and WbGST ($P < 0.0003$). Pre-incubation of sera from cockroach allergic subjects with WbGST partially depleted (by 50 to 70%) anti-Bla g 5 IgE, IgG, and IgG4 Abs. IgE epitope mapping of Bla g 5 revealed that two linear N-terminal epitopes are highly conserved in WbGST corresponding to Bla g 5 peptides partially involved in the inhibition of WbGST binding. Finally,

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mice infected with *Hb* developed anti-HbGST IgE and showed immediate type skin test reactivity to Bla g 5.

Conclusion—These data demonstrate that helminth GST and the aeroallergen Bla g 5 share epitopes that can induce allergic cross-sensitization.

Keywords

GST; cockroach; allergy; hygiene hypothesis; crossreactivity; filariasis

INTRODUCTION

Epidemiologic and experimental evidence suggests that helminth infections have a general ability to modulate allergic disease [1–5] through both parasite-induced IL-10 and the expansion of natural regulatory T cells (nTregs) [6–9]. Other studies, however, have shown that helminth infections may be associated with increased allergic manifestations [10–12], perhaps related to the Th2-dominated environment induced by the parasites [13] and/or the potential crossreactivity among allergens and helminth proteins [14]. Indeed, crossreactivity between helminth tropomyosin and allergenic tropomyosin from mites and cockroaches has been described [15–17]. Although crossreactivity among tropomyosins has dominated the discussion about helminth-aeroallergen crossreactivity [18], several other allergens have orthologues in helminths [19] that may be involved in cross sensitization.

In the present study, we investigated the crossreactivity among allergenic glutathione S-transferases (GST) and those in pathogenic helminths. GSTs play a variety of physiologic roles including detoxification of toxins, protection from oxidative stress, and eicosanoid metabolism [20]. The cytosolic GSTs can be subdivided into different classes (alpha, mu, pi, sigma, delta, nu, and others) each of which contain two basic structural motifs: an N-terminal thioredoxin motif and a C-terminal GST motif that is involved in formation of the H-site (a hydrophobic pocket that binds substrate). The sequence variability of the C-terminal GST motif confers species specificity to the molecule, making it a target of drug development for allergic diseases, cancer treatment, and parasitic infections [20] including vaccines.

Although GSTs are important helminth vaccine candidates [21–23], GSTs are known to be strong inducers of IgE. For example, the GST of the cockroach *Blattella germanica* (Bla g 5) is a major cockroach allergen, being an allergen inducing IgE in 30% to 71% of those with cockroach allergies [24–26]. Moreover, GST is a common allergen of house dust mite (HDM) — with IgE anti-GST being found in up to 96% of HDM allergic populations [27, 28].

The present study demonstrates marked similarities at the amino acid (aa) and structural level between filarial (and other helminth) GST and the major cockroach allergen Bla g 5. We have mapped the linear crossreactive epitopes in humans and have shown their involvement in the structural basis for this crossreactivity. Moreover, we have used mice infected with intestinal nematode *Heligmosomoides bakeri* (Hb) to demonstrate unequivocally that helminth infections induce parasite-specific IgE that sensitizes mice to Bla g 5. These data suggest that helminth infection promotes cross-sensitization to common allergens through molecular mimicry.

METHODS

Patients and sera

Sera from well characterized filaria-infected (Fil^+) individuals were utilized in this study. All patients were seen by the Clinical Parasitology Section of the Laboratory of Parasitic

Diseases under protocols approved by the Institutional Review Board of the NIAID and registered (NCT00001230; NCT00001645). The Fil^+ group in this study was composed of 47 patients with *Loa loa* (n=37), *O. volvulus* (n=6), or *W. bancrofti* (n=4). Among the 47, 39 were temporary residents of or travelers to filarial-endemic regions, while 8 were indigenous to these same regions. Sera from 29 filaria-uninfected (Fil^-; normal) individuals were obtained from the Department of Transfusion Medicine, Clinical Center, NIH, under protocols approved by the Clinical Center, NIH IRB.

All sera were tested for IgE to common allergens using Phadiatop® technology (Phadia, Uppsala, Sweden). Serum samples with levels below 0.35 kUA/l were considered negative and categorized as non-atopic. Sera from Phadiatop®-positive subjects were further tested for cockroach-specific IgE using an Immunocap™ assay specific for *Blattella germanica* (Bla g) (Phadia). Individuals positive for Bla g(levels above 0.35 kUA/l) were considered atopic for cockroach. Based on these data, the 76 subjects were divided into four groups based on their cockroach allergy and filarial infection status: 1) Fil^- and non-atopic, Ni–NA; $n = 15$ individuals; 2) Fil^- and atopic, Ni–A; $n = 14$; 3) Fil^+ and non-atopic, Fil+NA; $n = 11$; and 4) Fil^+ and atopic, Fil+A; $n = 36$.

Antigens and peptides

cDNA encoding the GSTs of *B. germanica* (Bla g 5) or *W. bancrofti* (WbGST) were cloned into bacmids. Transformed baculoviruses were used to infect Hi5 cells for expression of Bla g 5 or WbGST. Cell lysates and supernatants were purified on glutathione columns. The purities of Bla g 5 and WbGST were assessed by SDS-PAGE. Some experiments utilized recombinant Bla g 5 purchased from Indoor Biotechnologies Inc. (Charlottesville, VA). Recombinant Bla g 4 was purchased from Indoor Biotechnologies Inc.

A library of 40 peptides with purities greater than 80% that spanned the entire length of Bla g 5 (Table E1) was synthesized by Mimotope. Each peptide was 15 aa residues in length and overlapped adjoining peptides by 10 residues. Peptide #31 could not be purified and was not used in the experiments. Peptides were dissolved in HPLC-grade DMSO (Fisher Scientific, Pittsburg, PA) at 10 mg/ml to produce stock solutions and were kept at −40°C until used.

Epitope mapping

An array of 39 overlapping peptides and the full-lengh Bla g 5 was blotted onto PVDF membranes using a 96-well mini-fold dot blotter (Schleicher & Schuell, Inc., Riviera Beach , FL). After vacuum aspiration, air drying and blocking, membranes were incubated with six positive and two negative sera, depleted of IgG by incubation with protein G beads (GE Biotechnologies, Piscataway, NJ), diluted 1:25 in PBS-milk 5%. Membranes were then incubated with goat anti-human IgE (R&D, Minneapolis, MN), followed by peroxidase-conjugated rabbit anti-goat IgG, or peroxidase-conjugated anti-human IgG (Jackson ImmunoResearch, West Grove, PA) and developed with enhanced chemiluminescence substrate (Amersham- GE Biotechnologies, Piscataway, NJ) according to the manufacture's recommendations.

ELISA for Bla g 5 and WbGST

Measurements of Bla g 5, Bla g 4 and WbGST Abs were performed by ELISA as previously described [16]. Geometric mean (GM) + 3 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 Bla g 5, Bla g 4 and to WbGST.

Depletion ELISA

Depletion ELISA was performed as previously described [16] using Bla g 5, WbGST, or a nonrelated recombinant antigen (OvTropomyosin) to capture specific antibodies and coated plates to measure the level of depletion.

Inhibition ELISA

Sera samples from infected and non-infected subjects that were IgE and IgG positive for Bla g 5 and WbGST were incubated overnight with 20 μg in 0.1 mL of selected peptides (Pep 2, Pep 7, Peps 12–13, Peps 22–23, and Peps 27–29) dissolved in DMSO (diluted in 1% BSA/PBS from 10 mg/mL stock). Full-length Bla g 5, or WbGST in DMSO at 2% v/v was used as positive controls and 2% DMSO was used as negative control. The following day sera were transferred to plates coated with Bla g 5 or WbGST and developed for IgE Ab reactivity. To increase the sensitivity of the IgE-based assays, these sera were depleted of IgG by protein-G incubation (GE Healthcare, Piscataway, NJ).

3-D modeling

3-D structural models of Bla g 5 and WbGST were constructed by homology modeling with GST sigma from *Drosophila melanogaster* (DmGST1-1; Acc. No P41043, PDB 1m0u) [29] and GST of *O. volvulus* (OvGST2; Acc. No P46427, PDB 1tu7) [30] using the Protein Model Portal (http://www.proteinmodelportal.org/) [31]. The 3-D crystal structure of *H. bakery* GST 2 (HbGST2; PDB 1tw9) [32] was used to compare with the models predicted for Bla g 5 and WbGST (Fig E2 and Table E3). Molecular visualization of each model and 3-D alignment were performed using Swiss-pdb viewer (http://www.expasy.org/spdbv/).

Sequence alignment and phylogenetic tree

Lasergene MegAlign (DNAStar, Inc.) was used to compare the sequences of WbGST, Bla g 5, and HbGST2, and with themselves or with the protein sequences from other GST allergens of HDMs, cockroaches, and fungus (Table E4). Pair-wise or multiple-sequence alignments of the GSTs (Table E4) were performed using ClustalW (algorithmic Gonet series) and used to construct the phylogenetic tree. Bootstrap values were calculated using 1000 bootstrap repetition and 111 random seeds.

Mouse infection and skin testing

BALB/c female mice, 6–8 wk old, were purchased from Jackson Laboratories, housed at an Association for the Assessment and Accreditation of Laboratory Animal Care-approved facility at the National Institute of Allergy and Infectious Diseases (NIAID) and studied under an animal study proposal approved by the NIAID Animal Care and Use Committee. Mice were inoculated per os using a gavage tube with 200 infective third-stage larvae (L3) of Hb. Uninfected control mice (Ni) and mice infected with Hb for 30 d were skin tested. Mouse ear swelling assays [33] with modifications was performed to evaluate skin sensitivity. Mice were injected with 10 μg/10 μl of Bla g 4 (right ear) or Bla g 5 (left ear) and ear thickness measured with a caliper before and 15, 30, and 60 min after allergen injection. Alternatively, mice were injected i.v. with 200 μl of Evans' Blue solution (0.5%w/vol) 3 min after intradermal injection of the allergens and euthanized 15 min later. Ears were collected and the Evans' Blue dye extracted in formamide overnight at 63°C and quantified in a spectrophotometer at 620 nm.

Statistical analysis

GraphPad Prism v5.0 (GraphPad Software Inc., San Diego, California) was used for all of the statistical analyses (one-tailed Fisher's exact test, relative risks [RR] and odds ratios

[OR] with confidence intervals [CI], Spearman rank correlation and Wilcoxon matched-pairs signed rank test).

RESULTS

Bla g 5 and WbGST display related sequence and structure

We investigated the similarities between the cockroach GST allergen (Bla g 5) and a filarial GST from *W. bancrofti* (Fig 1). The sequences of Bla g 5 and WbGST were found to be closely related (30% identical, 47% similar) (Fig 1, A). Importantly, the N-terminal region of Bla g 5 and WbGST had a 41% identity over an 80-aa window, which classify them as "likely to crossreact" (WHO criteria [34]). Phylogenetic analysis that included multiple allergenic GSTs demonstrated that the WbGST clusters closely with Bla g 5 (Fig 1, B). Moreover, the predicted 3-D structures of Bla g 5 and WbGST showed that both GSTs—although from different GST families (sigma and pi)—had well conserved structures, especially in the N-terminal domain (Fig 1, C).

Abs to Bla g 5 crossreact with WbGST

To address the crossreactivity between filarial GST and Bla g 5, sera from filaria-positive (Fil^+) and filaria-negative (Fil^-) individuals were subdivided into cockroach allergic or non-allergic based on specific reactivity to cockroach (*B. germanica*) extract giving four groups: 1) Fil^-, non-atopic (Ni-NA); 2) Fil^-, atopic (Ni-A); 3) Fil^+, non-atopic (Fil+NA); and 4) Fil^+, atopic (Fil+A). Levels of GST-specific IgE, IgG, and IgG4 were then assessed (Fig 2, A). As can be seen, filarial infection (irrespective of atopic status) was associated with increased levels of IgE, IgG, and IgG4 Abs to both WbGST and Bla g 5. When the prevalence of Bla g 5-specific Abs was assessed among the atopic individuals (Table I), the presence of filarial infection was associated with increased reactivity to Bla g 5. Indeed, the prevalence of GST-specific IgE was 28.6% (4/14) in the Ni-A group compared with 63.9% (23/36) in the Fil+A group ($P = 0.026$). For the anti-GST IgG, the prevalence increased from 14.3% (2/14) to 69.4% (25/36) ($P = 0.0005$), and for anti-GST IgG4, there was an increase in prevalence from 14.3% (2/14) to 45% (17/36) ($P = 0.0302$) when Ni-A and Fil+A were compared (Table I). Thus, the Fil^+ individuals had significantly increased likelihoods of being positive for Bla g 5-specific IgE (RR of 2.9; OR = 4.4) and IgG (RR of 7.0; OR = 13.6) and IgG4 (OR = 5.3) (Table I). When similar analysis was performed for Bla g 4 (a different cockroach allergen that does not have a parasite orthologue) no significant association between infection status and antibody levels was found. For example: Ni-A and Fil+A showed 8.3% and 31% of anti-Bla g 4 IgE prevalence (P=0.102), respectively, and 8.3% and 25% (P=0.199) for IgG. Furthermore, a positive correlation was found between levels of the IgE, IgG, and IgG4 anti-WbGST Abs and the respective levels of anti-Bla g 5 (IgE, r = 0.38 and $P = 0.0003$; IgG, r = 0.68 and $P < 0.0001$; and IgG4, r = 0.52 and $P < 0.0001$) (Fig 2, B).

To test more specifically the crossreactivity between the two GST proteins, depletion analyses were performed (Fig 3). Pre-incubation of cockroach GST positive sera with WbGST was able to deplete 70% of Bla g 5-specific IgE, IgG, and IgG4, whereas pre-incubation with a recombinant, structurally unrelated filarial control protein known also to be allergenic (OvTropomyosin) [16] failed to deplete Bla g 5-specific Abs. Incubation of sera with full-length Bla g 5 or WbGST was able to inhibit the IgE binding to both proteins, but the data suggested that the cross-reactivity was not complete (data not shown).

Involvement of two N-terminal linear epitopes in crossreactivity between Bla g 5 and WbGST

To investigate if linear epitopes were involved in the molecular basis of Bla g 5/WbGST crossreactivity, overlapping peptides (15 mers) covering the full length of Bla g 5 (Table E1) were used to map the epitopes involved in IgE and IgG binding (Fig 4) with sera of 4 filaria-infected (S#1,2,5 and 6) and 2 non-infected subjects (S#3 and 4). As seen, four linear regions of Bla g 5 were identified that were reactive to both IgG and IgE: two regions in the N-terminal domain (peptide 7, aa residues 31–45 and peptides 12–13, aa residues 56–75) and two regions in the C-terminal domain (peptides 22–23, aa residues 106–125 and peptides 27–29, aa residues 131–155). Three of the regions (Pep 7, Peps 12–13, and Peps 22–23) corresponded to exposed regions of Bla g 5 that were predicted to be antigenic (Fig 4, B). There was strong reactivity to Pep 7 in 3/6 for IgE and 4/6 positive for IgG (Fig 4, A). Four of six had a weak signal to Peps 12–13 for IgE, but for IgG there was strong reactivity. The region of the molecule corresponding to Peps 22–23, predicted to be highly antigenic (Fig 4, B), had weak but measurable reactivity for IgE (in 3/6) and IgG (in 4/6) (Fig 4, A). Surprisingly, there was consistent binding of IgE (2/6) and IgG (6/6) to Peps 27–29, a region predicted to be poorly antigenic. We next mapped the four antigenic regions to 3-D models of Bla g 5 (Fig E1 and Table E2) and compared them to the corresponding regions in the 3-D model of WbGST (Fig E2 and Table E3). Peptides 7 and 12–13 were found to be highly conserved between Bla g 5 and WbGST with 40% sequence identity and Carbon-alpha root square mean difference of 2.4 Å and 1.7 Å, respectively, at the structural level.

Each of the antigenic peptides was clearly able to partially inhibit the binding of IgE from infected individuals to Bla g 5 (Fig 5A). For example, peptide 7, peptides 12–13, peptides 22–23 and peptides 27–29 were able to inhibit the binding of IgE to plate immobilized Bla g 5 ($P<0.04$). In contrast, peptide 2 that was not recognized by any of the subjects tested could not inhibit binding at all. The fact the peptides could not inhibit completely IgE binding to Bla g 5 suggests that both non-linear conformational and linear (possibly conformational) epitopes are involved in the IgE response to this allergen. In the present study, we examined the role played by these linear regions in the cross-reactivity to WbGST. When the peptides were used to inhibit the WbGST ELISA, only peptide 7 and peptides 12–13 could inhibit IgE binding suggesting that the other peptides are not relevant for cross-reactivity. Relevant non-linear conformational epitopes could not be evaluated in this study.

Conversely, using sera from filaria-infected patients (Fig E3) or Bla g sensitized mice (ip immunizations) (Fig E4) in an inhibition ELISA to verify the specificity of the cross-reactivity, we were able to show that peptides from WbGST and HpGST-2 corresponding to the Bla g 5 peptides 7 and 12–13 could inhibit anti-Bla g 5 IgE equally well (Fig E3 and E4). In addition, Bla g 5 and HpGST peptides could also inhibit the WbGST human IgE ELISA (Fig E3B).

Helminth infection can induce cross-sensitization in mice

To assess whether helminth infection could induce crossreactive IgE and cause cross-sensitization in vivo, we infected BALB/c mice with *Heligmosomoides bakeri* (Hb), a parasite that contains a GST (HpGST-2) that is 32% identical (50% similar) to Bla g 5 and contains conserved sequences to the linear epitopes mapped for Bla g 5 (Fig E2 and Table E3), and likely also shows conserved conformational epitopes. More importantly, mice infected with Hb developed IgE to Bla g 5 (and not to an unrelated cockroach antigen, Bla g 4) (Fig 6, A); further this IgE mediated immediate-type hypersensitivity responses as measured intradermally administered antigen (Bla g 5 in one ear or Bla g 4 in the contralateral ear). Hb-infected mice developed immediate hypersensitivity reactions to Bla g 5 but not to Bla g 4 (Fig 6, B). To confirm this finding, skin-tested animals were injected

with Evans' blue dye i.v. 3–5 min after intradermal injection, and dermal edema was assessed by dye leakage (Fig 6, C). As seen, Hb-infected mice challenged with Bla g 5 had clear evidence of vascular leakage, whereas Bla g 4 (in the context of Hb infection) failed to induce an immediate hypersensitivity reaction. This was not due to a failure of Bla g 4 to induce sensitization, as mice sensitized (by i.p. immunizations) to either Bla g 4 or Bla g 5 could mount an immediate hypersensitivity response to the homologous antigen challenge (data not shown). As can also be observed in Fig 6, C, uninfected (Ni) mice reacted to neither Bla g 5 nor Bla g 4.

DISCUSSION

Although the "Hygiene Hypothesis" [35] suggests that a decrease in helminth (and/or other) infections may be responsible for the global increase in allergic diseases, there are data to show helminth infection can actually increase the risk for atopic disease [10–12] and asthma [35–38]. One concept that has emerged—in an attempt to understand the allergy-promoting capacity of certain helminth infections—has been the induction of parasite-specific IgE responses that can crossreact with allergens (particularly aeroallergens), thereby inducing allergic effector responses [14–16, 19, 39].

In regions of the world that are highly endemic for parasitic worm infections, cockroach and HDM are responsible for the overwhelming majority of aeroallergen sensitization [10, 11, 40, 41]. Among the 10 defined allergens of cockroach in the WHO/OIUIS Allergen Nomenclature Database (www.allergen.org), GSTs (especially Bla g 5) are among the important cockroach allergens [24, 42]. Although the GSTs studied here belong to different families—Bla g 5 is a GST sigma, while WbGST and HbGST2 are GSTs pi and nu—they are evolutionarily related [43]. Immunologic crossreactivity for GSTs of different families that are evolutionarily conserved, although considered to be rare [43, 44], has been demonstrated for Der p 8 (a HDM GST mu) and Bla g 5 (GST sigma) [27]. We found by in silico analysis of Bla g 5 and WbGST that the N-terminal motif of WbGST displayed 41% identity over an 80-aa residue span, fulfilling the WHO paradigm used to predict crossreactivity [34]. In addition, the 3-D modeling onto which the allergenic linear epitopes were mapped (Fig E2) provided further molecular and structural basis for the crossreaction seen.

Aeroallergens such as Bla g 5, in contrast with food allergens that tend to have mostly linear epitopes due to proteolytic digestion of the antigen, have primarily conformational epitopes displayed on the surface of the protein. Of note, some linear epitopes can also be conformational if that sequence is surface-exposed [45], and, perhaps, this is the case of the linear epitopes identified in this study for Bla g 5. Because the peptides identified as IgE epitopes were unable to completely inhibit IgE binding to Bla g or WbGST, our data suggests that conformational epitopes are quite important, as suggested by the difference in the potency of ELISA inhibition between the entire protein and the peptides. Nevertheless our results suggest that the N-terminal portion of Bla g 5, the most conserved region of the molecule, was likely to be the primary regioninvolved in the crossreactivity to WbGST. These data agree with data from epitope mapping of the bovine filarial parasite *Setaria cervi* GST that has recently shown that the N-terminal end was highly immunogenic [46]. Because our data suggest only partial crossreactivity (i.e., WbGST could not inhibit completely anti-Bla g 5 Abs), we conclude that Bla g 5-specific Abs are comprised of both crossreactive and non-crossreactive Abs. The non-crossreactive Abs may be directed to a less conserved portion of the molecules or may reflect small differences in the peptide structure or in non-linear conformational epitopes.

The most important finding in the present study was the demonstration that this induction of crossreactive, allergen-specific IgE could be recapitulated in vivo using a model of a rodent

gastrointestinal nematode in BALB/c mice. We were able to show that this helminth infection induced crossreactive anti-Bla g 5 IgE that mediated skin sensitivity (immediate hypersensitivity reactions) to Bla g 5, but not to Bla g 4 (a non-orthologous cockroach allergen). The use of Bla g 4 was important to exclude the possibility of helminth-induced polyclonal IgE activation through a Th2-skewed immune response that could induce indiscriminate allergic sensitization [47, 48]. Our data suggest that cross-sensitization was based primarily on the identity/similarity of the proteins of the parasite to their aeroallergen orthologues.

Cross-sensitization may have important implications for understanding the hygiene hypothesis [16] and to helminth-associated allergy [14]. We show that parasite can induce crossreactive IgE with possible implications of cross-sensitization in vivo that may have significant implications for vaccine development against helminth parasites as suggested by the allergic reactivity (related to the presence of pre-existing IgE to the vaccine protein) to a first generation hookworm vaccine [22]. Because GSTs are potential vaccine candidates for *S. mansoni* [21], *N. americanus* [22, 49] and *W. bancrofti* [23], not only is the presence of pre-existing vaccine-specific IgE of concern, but also is the potential for cross reactions to aeroallergen (e.g. cockroach) orthologues.

Together, our data demonstrating induction of crossreactive allergen-specific Abs by helminth infection in both humans and experimentally infected mice clearly help to understand the conflicting data that have emerged in the study of the helminth/allergy interface. Not only have we provided a structural basis for the induction of crossreactive Abs, but we have also identified concerns that must be addressed in terms of allergy epidemiology, vaccine development [22] and therapeutic approaches to allergic diseases.

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Abbreviations used

Bla g 5	GST of the cockroach *Blattella germanica*
CI	confidence interval
Bla g	*Blattella germanica*
ELISA	enzyme-linked immunosorbent assay
Fil$^+$	filaria-infected
Fil$^-$	filaria-uninfected
Fil-A	Fil$^+$ and atopic
Fil-NA	Fil$^+$ and non-atopic
GM	geometric mean
GST	glutathione-S transferase
Hb	*Heligmosomoides bakeri*
HDM	house dust mite
HbGST-2	GST of *H. bakeri*

IQR	interquartile range
Ni-A	Fil$^-$ and atopic
Ni-NA	Fil$^-$ and non-atopic
nTreg	natural regulatory T cell
OR	odds ratio
SmGST	GST of *Schistosoma mansoni*
WbGST	GST of *Wuchereria bancrofti*
RR	relative risk

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Key message

- Cockroach and helminth GST crossreact because of remarkable molecular and structural similarities
- Filarial infection is associated with increased prevalence of cross-reactive anti-Bla g 5 IgE with possible clinical implications
- Experimental infection with helminth induced cross-reactive allergy as measured by skin test
- Vaccine development for helminths should be concerned about the potential impact of crossreactivity with common allergens

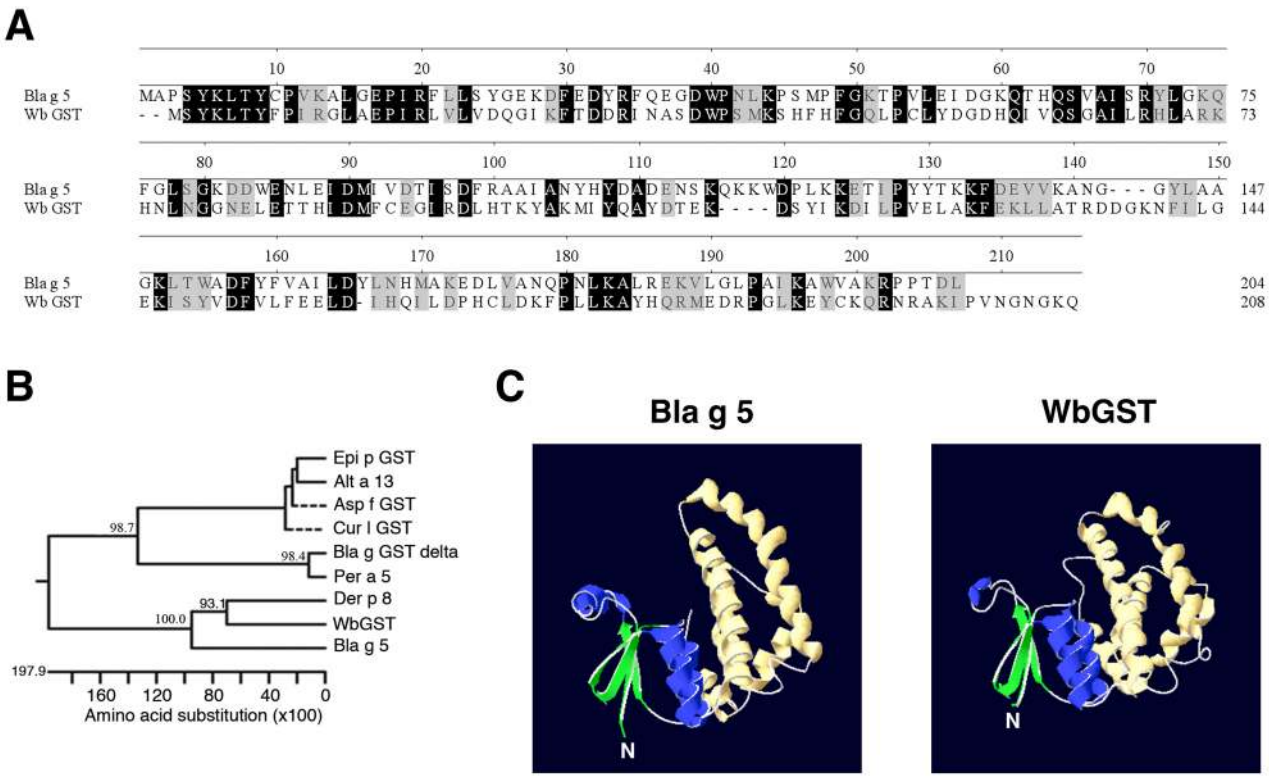

FIG 1.

Sequence and structural similarities between Bla g 5 and WbGST. **A,** Alignment of Bla g 5 and WbGST sequences showing identical (shaded in black) and similar (shaded in gray) amino acids. **B,** Phylogenetic tree of allergenic GSTs. **C,** Predicted 3-D structure of Bla g 5 and WbGST color-coding basic structure for comparison.

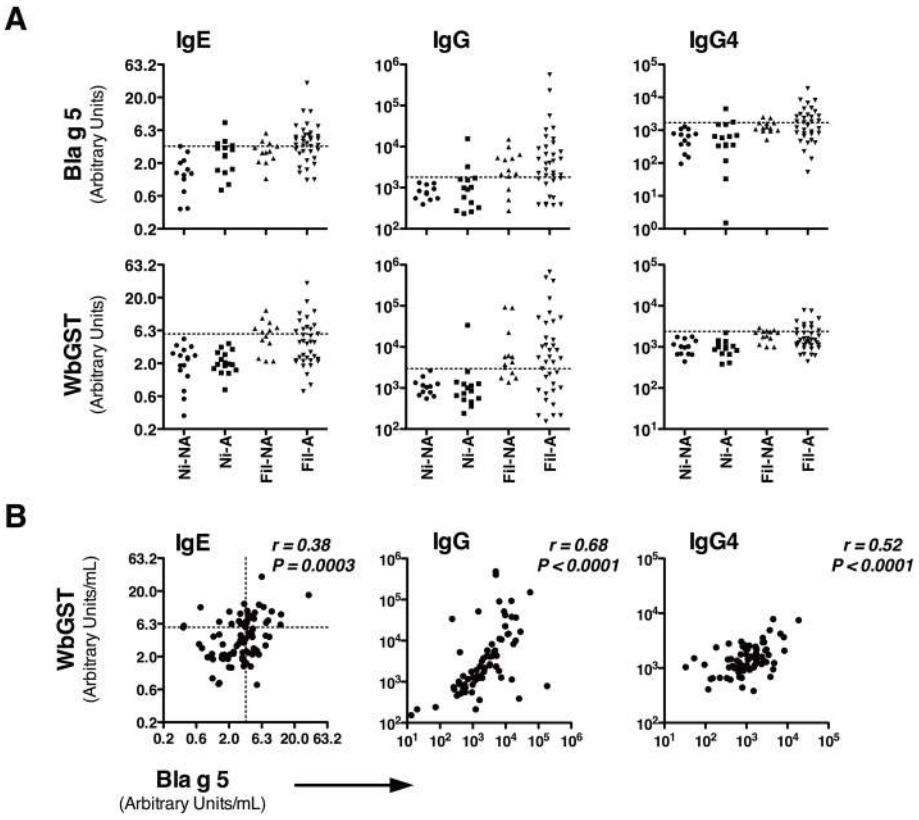

FIG 2.

Correlation between WbGST and Bla g 5 Ab levels. (**A**) Level of anti-Bla g 5 and anti-WbGST IgE, IgG, and IgG4 Abs in non-infected and non-atopic (Ni-NA), non-infected and atopic (Ni-A), filaria-infected and non-atopic (Fil+NA) and filaria-infected and atopic (Fil +A) individuals. Individuals above the dotted lines (representing GM + 3 SD of Ni-NA) were considered positive for anti-Bla g 5 antibodies. B, Correlation between anti-WbGST and anti–Bla g 5 IgE, IgG, and IgG4; each point represents an individual. $n = 76$.

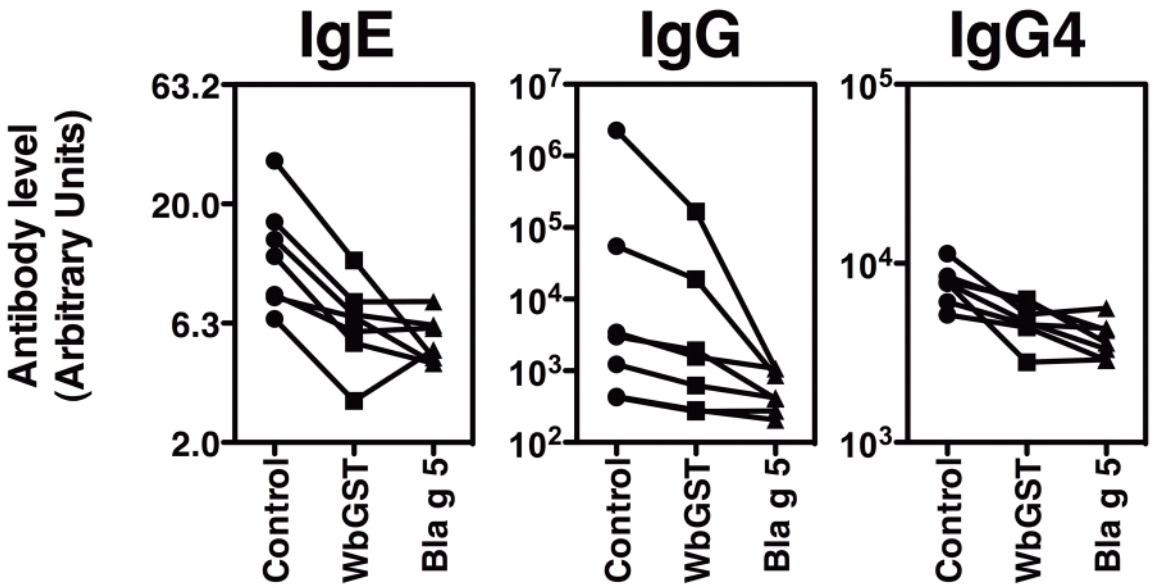

FIG 3.

WbGST-specific Abs crossreact with Bla g 5 due to conserved epitopes. Sera of patients positive for anti-Bla g 5 IgE, IgG, and IgG4 were incubated in plates coated with a control antigen (OvTropomyosin), WbGST or Bla g 5. Following incubation, sera were transferred to Bla g 5-coated plates and developed for IgE, IgG, and IgG4. Each dot represents one individual.

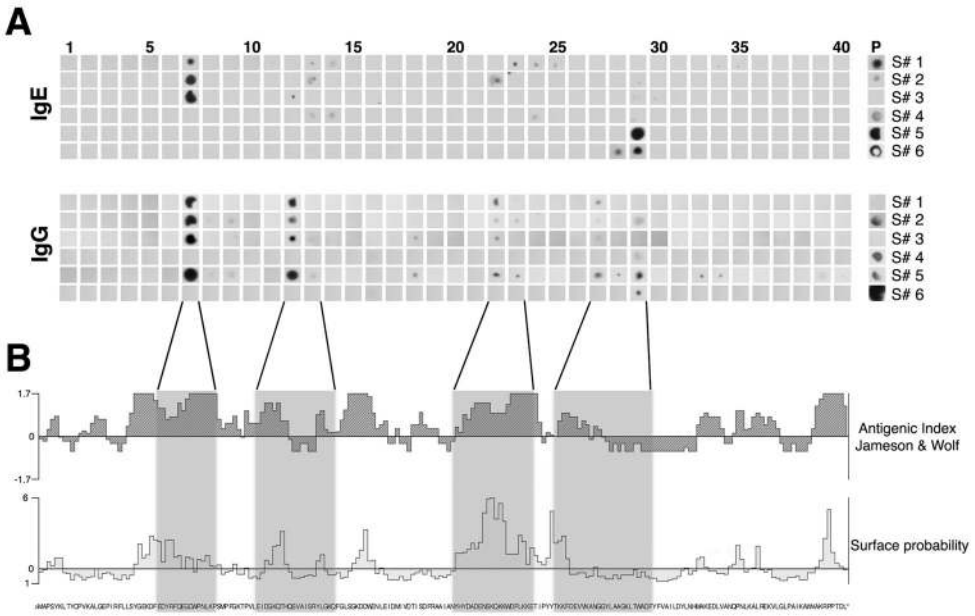

FIG 4.

Linear epitope mapping of Bla g 5. **(A)** Thirty-nine overlapping peptides spanning the entire sequence of Bla g 5 was used to perform western dot blots for IgE an IgG binding epitopes (numbers reflect peptide identity, S stands for serum and P for recombinant Bla g 5). **(B),** The results were compared to in silico epitope prediction based on the Bla g 5 sequence.

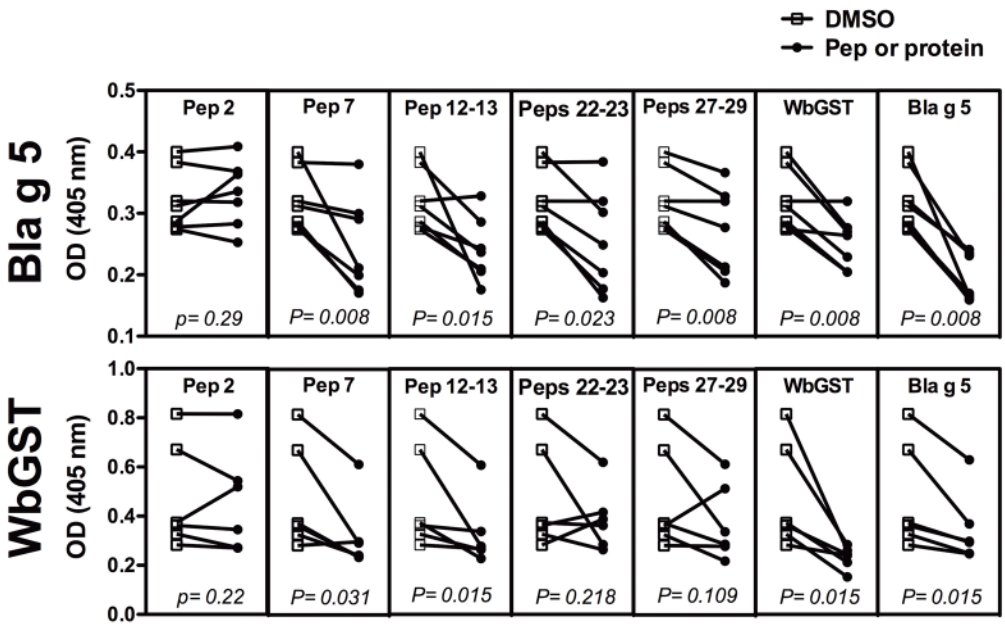

FIG 5.

Antigenic peptides can inhibit IgE binding to Bla g 5 and WbGST. Sera from infected patients positive for anti-Bla g 5 and anti-WbGST Abs were incubated with DMSO (open squares) or specific peptides or recombinant proteins (closed circles) and then transferred to Bla g 5- or WbGST-coated multiwell plates. Optical densities (OD) are shown for all sera tested. Differences were compared using the Wilcoxon matched-signed rank test.

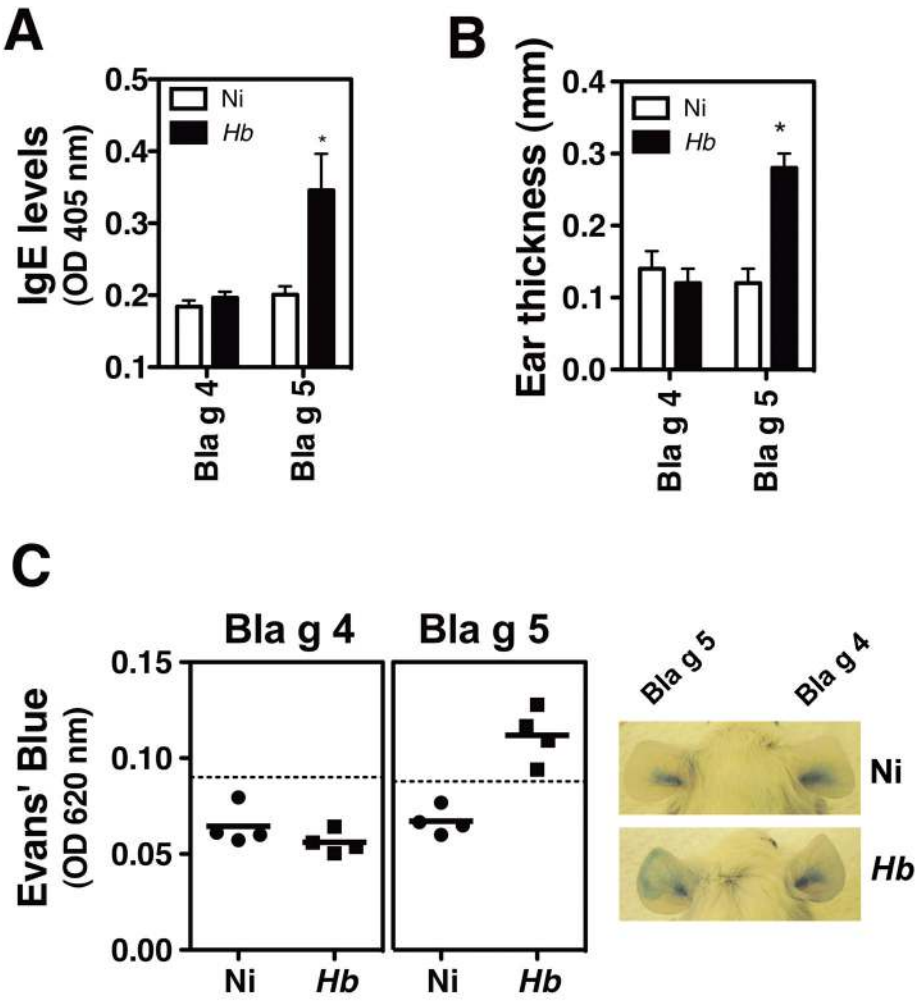

FIG 6.

Helminth infection can cause cross-sensitization to Bla g 5. Mice infected with *Heligmosomoides bakeri* (Hb) developed IgE to Bla g 5 but not to Bla g 4 as measured by ELISA **(A)** and skin test reactivity by ear thickness **(B)** or by Evans' blue dye extravasation **(C)**. Data represent one of four experiments performed ($n = 4–5$ per group per experiment). Bars (in A and B) represent means ± SE. In Panel C, each dot represents one animal and the dotted lines represent the GM of the Ni group + 3 SD.

TABLE I

Filarial infection is associated with higher prevalences of anti-Bla g 5 antibodies

	Prevalence (%) *		RR (CI)	Odds ratio (CI)	*P* value †
	Ni–A ‡	Fil+A §			
IgE	28.6	63.9	2.9 (1.06–8.12)	4.4 (1.15–17.0)	0.0262
IgG	14.3	69.4	7.0 (1.75–28.3)	13.6 (2.60–71.5)	0.0005
IgG4	14.3	47.2	3.6 (0.92–14.7)	5.3 (1.05–27.5)	0.0302

* Prevalence was calculated using cut-off values as explained in Fig 2.

† *P* value was calculated using one-sided Fisher's exact test.

‡ Ni–A, non-infected and atopic; $n = 14$.

§ Fil+A, filaria-infected and atopic; $n = 36$.