

Evolutionary immune response to conserved domains in parasites and aeroallergens

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ABSTRACT

The immune response based on immunoglobulin E (IgE) evolved as a defense against specific parasitic infections. In the absence of active helminthic infections, the immune system has redirected its IgE epitopes toward innocuous environmental antigens. Helminths and aeroallergens have a similar stereotypical IgE response to unique antigens that can not be explained by chance alone. This study was designed to evaluate potential homology between conserved protein domains embedded in parasitic organisms and aeroallergens. Search and retrieval systems for nucleotide and protein sequences (Entrez, BLAST, and National Center for Biotechnology Information) were searched to identify conserved domains between allergens and certain parasites. A total score was developed that correlated positively with homology between compared sequences. Over 2000 domains were examined. We found matches with a high total score (>100) that signified a strong positive correlation between sequences in allergens ($n = 30$) and parasites ($n = 13$). Multiple shared conserved domains were identified between parasites and allergens. Parasite–allergen combinations with the most significant homology (greatest total score) were *Plasmodium falciparum* enolase and *Hev b9* (total score, 612), *Schistosoma mansoni* albumin and *Fel d 2* (total score, 991), *Ascaris lumbricoides* tropomyosin and *Ani s3* (total score, 531), and *Wuchereria bancrofti* trypsin and *Blo t3* (138). Homologous conserved domains exist in specific parasites and allergens, consistent with the theory that the human IgE–eosinophil immune response to common allergens is a direct consequence of stimulation by parasitic organisms.

(Allergy Asthma Proc 34:93–102, 2013; doi: 10.2500/aap.2013.34.3616)

The International Study of Asthma and Allergies in Childhood elucidated the global prevalence of atopic disorders among different geographic populations.^{1,2} A direct relation exists between individual parasitic burdens and the blunting of the allergic response. However, allergies increase with antiparasitic treatment,³ suggesting an intersection between parasitic infections and allergies at the immunoglobulin E (IgE)–Th2 lymphocyte–eosinophil response.^{4–7}

The hygiene hypothesis suggests an inverse relation between allergenicity and exposure to various infectious agents or bacterial endotoxins.^{8,9} This hypothesis suggests that an inverse relationship exists between microbial exposure during childhood (such as from farm animals, domestic pets, day care attendance, and large family size) and atopic diseases; a greater exposure to microbes is associated with lower probability of developing diseases such as hay fever and asthma. This may be explained by the allergy being mediated primarily by the interaction of B lymphocytes, IgE, and

mast cells, as controlled by the Th2 response. The hygiene hypothesis has been extended to include microbial exposure and a greater number and chronicity of acquired infections.¹⁰ A lower frequency of allergies has been reported in developing countries, suggesting that the multiplicity of childhood infections protects against allergic sensitization by inducing an immunologic shift from a predominant Th2 response to a Th1 response.¹⁰

Bacterial and viral infections typically promote protective Th1 cytokines. Th2 and Th1 lymphocyte responses are counterregulatory, and reduced Th1 could promote the development of Th2 responses to antigens in the environment.¹¹ Repeated exposure to infections may shift the body from a primarily Th2 response to a Th1 response. However, a deviation toward Th1 responses from microbial product exposure does not fully explain all aspects of the hygiene hypothesis.

Similar to the hygiene hypothesis, there is a negative relation between helminthic exposure and the prevalence of childhood asthma and atopic responses. The Th2 immune response is the primary underlying mechanism that generates atopic disease, and it also is the immune response that is directed against helminthic infections.^{12–15} In the evolution of the immune system, with its initial function to assist in protection against parasites such as *Schistosoma*, a storage depot of conserved domains had been generated for future use by the human immune system when it should encounter the same parasite again. However, with fewer parasites

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Published online November 2, 2012

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to fight, the dormant IgE genetic code for various epitopes redirects itself toward other antigens, such as innocuous environmental antigens or aeroallergens.

Helminths and aeroallergens have a similar stereotypical IgE response to unique antigens of specific proteins that can not be explained by chance alone. An evolutionarily conserved mechanism for selecting particular motifs likely exists. Recent epidemiological studies have suggested such cross-reactivity against parasites by strongly induced Th2-type responses, including high levels of IgE, circulating eosinophils, and mast cells.^{9,16–20}

In the present study, we investigated the common domains that exist between species that stimulate the Th2 pathway response, including parasites and modern day allergens. Protein sequences from known allergens were compared with sequences from tropical and more temperate parasites and examined for areas of homology for possible IgE binding. We showed that the human IgE response to the selected helminthic enzymes was responsible for allergy to many common human environmental allergens because of homologous conserved domains.

MATERIALS AND METHODS

Allergen Selection

Twenty-eight aeroallergens with a large number of homologous protein sequences across different families and phyla were selected (Table 1).

Sequence Analysis

Each allergen sequence was compared with a protein database of selected parasites from the National Center for Biotechnology Information²¹ (NCBI; www.blast.ncbi.nlm.nih.gov) using the Entrez and FASTA databases. Entrez Global Query was an integrated search and retrieval system that provided access to all databases simultaneously with a single query string and user interface²² (www.ncbi.nlm.nih.gov/sites/gquery). Entrez efficiently retrieved related sequences, structures, and references and provided views of gene and protein sequences and chromosome maps. FASTA, a DNA and protein sequence alignment software program, was used with the Position-Specific Iterative Basic Local Alignment Search Tool (PSI-BLAST). FASTA was designed to measure protein sequence similarity (www.ncbi.nlm.nih.gov/BLAST/tutorial/Altschul-1.html).²³

The PSI-BLAST tool compared the entered amino acid sequences against hundreds of complete and incomplete protein databases. The PSI-BLAST program used a series of algorithms based on the Smith-Waterman model and examined sequence homology between two sequences. The algorithms searched for "high-scoring segment pairs" between sequences and

investigated the likelihood that the sequences could be related. The PSI-BLAST program generated a significance score (S score) and expect value score (E value) based on statistical significance, which were noted in this study. An algorithm generated the S score with higher numbers showing greater sequence homology. The E value represented the number of expected "hits" generated within PSI-BLAST that were expected by chance, which was based on the size of the database and sequence, and was analogous to background noise. A lower E value represented a more significant result and indicated a lower likelihood that the match was by chance. Therefore, the S score represented the relative alignment of the sequences with a high S score and a low E value being the most significant. For this study, an S score of >200 indicated a significant match between allergen and helminth, and an E value of <0.01 (equivalent to *p* value) ruled out a match by chance.

Each allergen sequence was compared with the known protein database of selected parasites. An E value was assigned to each sequence. The E value related to the level of sequence homology expected for a sequence of a given length. The E value was then used to develop a total score (T score). The E value was set at 10 for inclusion, with those E values found <0.001 to be likely significant for homology.

The T score, defined as the lower E value (<0.01) and higher S score (>200), was then recorded for each allergen–parasite pair. The value of the T score positively correlated with the homology between the two sequences. A T score of <40 essentially correlated with nonspecific E values. T scores of >100 signified E values with a strong homology between allergen sequence and parasite sequence.

Parasite Selection

Protein sequences for 13 parasites were selected against 28 aeroallergens. (Table 2)

RESULTS

Enolase

Enolase had significant homology with all helminths except *Strongyloides stercoralis* and *Ascaris lumbricoides*. These relations had high T scores (>100). The allergens studied included those from the fungi phyla (Alt a 6, Cla h 6, Cur l 2, Asp f 22, Pen c22, and Rho m 1) and enolase from the Plantae kingdom allergen, Hev b 9 (Fig. 1).

Albumin

Albumin comparisons showed significant homology between all allergens (Can f 3 [T score, 968], Fel d 2 [T score, 991], Bos d 6 [T score, 921] Equ c 3 [T score, 966],

Table 1 Aeroallergen selection

	Allergen	Scientific Name	Taxonomy	Allergen Type (common name)
Enolase	Alt a 6	<i>Alternaria alternata</i> *#	Fungi (mold)	<i>Alternaria</i> rot
	Cla h 6	<i>Cladosporium herbarum</i> *	Fungi (mold)	<i>Davidiella tassiana</i>
	Cur l 2	<i>Curvularia lunata</i> *	Fungi	—
	Asp f 22	<i>Aspergillus fumigatus</i>	Fungi (mold)	<i>Aspergillus</i>
	Pen c 22	<i>Penicillium citrinum</i>	Fungi (mold)	Penicillin
	ρ m 1	<i>Rhodotorula mucilaginosa</i>	Fungi (mold)	Yeast
	Hev b 9	<i>Hevea brasiliensis</i>	Plantae	Para rubber tree (latex)
Albumin	Can f 3	<i>Canis familiaris</i> (<i>Canis domesticus</i>)	Chordata	Dog
	Fel d 2	<i>Felis domesticus</i>	Chordata	Cat, saliva
	Bos d 6	<i>Bos domesticus</i>	Chordata	Domestic cattle§
	Equ c 3	<i>Equus caballus</i>	Chordata	Domestic horse
	Gal d 5	<i>Gallus domesticus</i>	Chordata	Domestic chicken
Tropomyosin	Ani s 3	<i>Anisakis simplex</i>	Nematoda	Roundworm
	Hel as 1	<i>Helix aspersa</i>	Mollusca	Brown garden snail§
	Tod p 1	<i>Todarodes pacificus</i>	Mollusca	Squid
	Blo t 10	<i>Blomia tropicalis</i>	Arthropoda	Tropical dust mite
	Der f 10	<i>Dermatophagoides farinae</i>	Arthropoda	American house-dust mite
	Der p 10	<i>Dermatophagoides pteronyssinus</i>	Arthropoda	European house-dust mite
	Lep d 10	<i>Lepidoglyphus destructor</i>	Arthropoda	Storage mite
	Pen m 1	<i>Penaeus monodon</i>	Arthropoda	Black tiger shrimp§
	Bla g 7	<i>Blattella germanica</i>	Arthropoda	German cockroach
	Per a 7	<i>Periplaneta americana</i>	Arthropoda	American cockroach
	χ k 10	<i>Chironomus kiiensis</i>	Arthropoda	Midge
	Lep s 1	<i>Lepisma saccharina</i>	Arthropoda	Silverfish
Trypsin	Blo t 3	<i>Blomia tropicalis</i>	Arthropoda	Tropical dust mite
	Der f 3	<i>Dermatophagoides farinae</i>	Arthropoda	American house-dust mite
	Der p 3	<i>Dermatophagoides pteronyssinus</i>	Arthropoda	European house-dust mite
	Eur m 3	<i>Euroglyphus maynei</i>	Arthropoda	Mayne's house-dust mite

Allergen protein sequences were downloaded from the International Union of Immunological Societies Allergen Nomenclature Sub-Committee (IUISANSC) database (www.Allergen.org) and chosen for analysis. The four allergens selected were enolase, albumin, tropomyosin, and trypsin. In analyzing the albumin group, sequences for nonallergenic albumins from nonmammalian species were incorporated in the selection for analysis and included *Taeniopygia guttata* (zebra finch), *Larus argentatus* (herring gull), and *Neoceratodus forsteri* (Australian lungfish). These particular species were selected to show that homologous domains exist across different phyla.

*Tropical fungal plant pathogen.

#This fungus lives on dead and decaying plant tissue in pistachio orchards and its spores contaminate pistachios in the orchard or during the handling process.

§Common foods in different geographic regions.

and Gal g 5 [498]) tested to the proto albumin protein *Schistosoma mansoni*. However, none of these modern day allergens displayed significant homology with any other parasites tested to albumin. The homology was calculated with *Mus musculus* (T score, 1074). *Neoceratodus forsteri* (Australian lungfish), *Taeniopygia guttata* (zebra finch), and *Larus argentatus* (herring gull) were entered into the NCBI's Protein BLAST protocol to see if the *S. mansoni* conserved domains exist outside mammals. All three animals showed significant homology, with T scores of 43, 103, and 117. No other parasites had proteins that showed homology to the aeroallergens tested (Fig. 2).

Tropomyosin

Tropomyosin was represented by 12 separate allergens from across three classes in kingdom Animalia (Arthropoda, Mollusca, and Nematoda). The Tod p 1 allergen, representing the *Todarodes pacificus* (squid in the Mollusca class), showed no significant homology with any of the parasites. The other 11 aeroallergens (Blo t 10, Der f 10, Der p 10, Lep d 10, Ani s 3, Bla g 7, Per a 7, Chi k 10, Lep s 1, Hel as 1, and Pen m 1) all showed significant homology, with T scores of >100 for *Onchocerca volvulus*, *S. mansoni*, *Schistosoma japonicum*, and *Clonorchis sinensis*. These included allergens

Table 2 Highly conserved domains among aeroallergens and parasites

Allergen	Ascaris lumbricooides	Clonorchis sinensis	Entamoeba histolytica	Fasciola hepatica	Leishmania braziliensis	Leishmania major	Leishmania donovani	Onchocerca volvulus	Plasmodium vivax	Plasmodium falciparum	Schistosoma mansoni	Schistosoma japonicum	Wuchereria bancrofti
Enolase													
Alt a 6	—	+	+	+	+	+	+	+	+	—	+	+	+
Cl a h 6	—	+	+	+	+	+	+	+	+	—	+	+	+
Cur l 2	—	+	+	+	+	+	+	+	+	—	+	+	+
Asp f 22	—	+	+++	+	+	+	+	+	+	—	+	+	+
Pen c 22	—	+	+++	+	+	+	+	+	+	—	+	+	+
ρ m 1	—	+	+	+++	+	+	+	+	+	+	+	+	+
Hev b 9	—	+++	+	+++	+	+	+	+	+++	+	+++	+	+
Can f 3	—	—	—	—	—	—	—	—	—	—	+++	—	—
Fel d 2	—	—	—	—	—	—	—	—	—	—	+++	—	—
Bos d 6	—	—	—	—	—	—	—	—	—	—	+++	—	—
Equ c 3	—	—	—	—	—	—	—	—	—	—	+++	—	—
Gal d 5	—	—	—	—	—	—	—	—	—	—	+++	—	—
Tropomyosin													
Ani s 3	+++	+	—	—	—	—	—	+++	—	—	+	—	—
Hel as 1	+	+++	—	—	—	—	—	+	—	—	+++	—	—
Tod p 1	+	+	—	—	—	—	—	+	—	—	+	—	—
Blot 10	+	+	—	—	—	—	—	+	—	—	+	—	—
Der f 10	+	+	—	—	—	—	—	+	—	—	+	—	—
Der p 10	+	+	—	—	—	—	—	+	—	—	+	—	—
Lep d 10	+	+	—	—	—	—	—	+	—	—	+	—	—
Pen m 1	+	+	—	—	—	—	—	+	—	—	+	—	—
Bl a g 7	+	+	—	—	—	—	—	+	—	—	+	—	—
Per a 7	+	+	—	—	—	—	—	+	—	—	+	—	—
X k 10	+	+	—	—	—	—	—	+	—	—	+	—	—
Lep s 1	+	+	—	—	—	—	—	+	—	—	+	—	—
Blot 3	—	—	—	—	+	+	—	+	+	+	+	+	+
Der f 3	—	—	—	—	—	—	—	—	—	—	+	—	—
Der p 3	—	—	—	—	—	—	—	—	—	—	+	—	—
Eur m 3	—	—	—	—	—	—	—	—	—	—	+	—	—

For comparison of homology between allergens and parasites, each of the chosen allergen sequences were compared against the same group of parasites. The parasites searched included global parasites and others endemic to specific geographic regions. The parasites included *Ascaris lumbricooides*, *Onchocerca volvulus*, *Schistosoma mansoni*, *Schistosoma japonicum*, *Clonorchis sinensis*, *Plasmodium falciparum*, *Plasmodium vivax*, *Leishmania major*, *Leishmania braziliensis*, *Fasciola hepatica*, *Wuchereria bancrofti*, *Strongyloides stercoralis*, and *Entamoeba histolytica*.

Allergen protein sequences were downloaded from the IUISANSC Internet site. The parasitic antigens were from several different organisms across different phyla. When the IUISANSC database had an allergen with >1 Universal Protein Resource sequence, all sequences were entered into the PSI-BLAST database with the strongest S score and E value chosen. A higher S score and E value indicated stronger homologous domains and an increased likelihood that two sequences would be identical. More than 100 allergens and their variants were searched using this protocol to yield the listed results. We chose to work with 3 of the 15 protein families with the highest number of allergens classified by source (plant, animal, fungi, or bacteria) and route of exposure (inhalation, ingestion, sting bite, or contact).

+ denotes significant homology (T score, >100).

+++ denotes highly significant homology (T score, >400).

IUISANSC = International Union of Immunological Societies Allergen Nomenclature Sub-Committee; PSI-BLAST = Position-Specific Iterative Basic Local Alignment Search Tool.

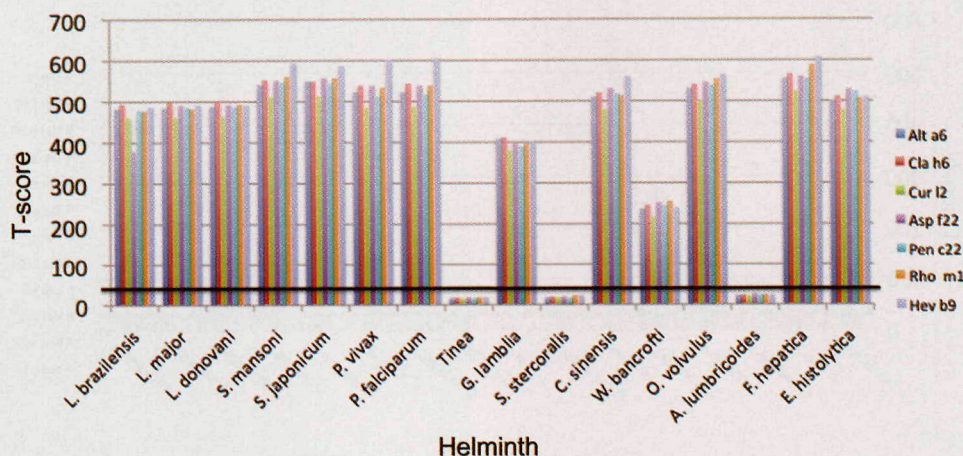
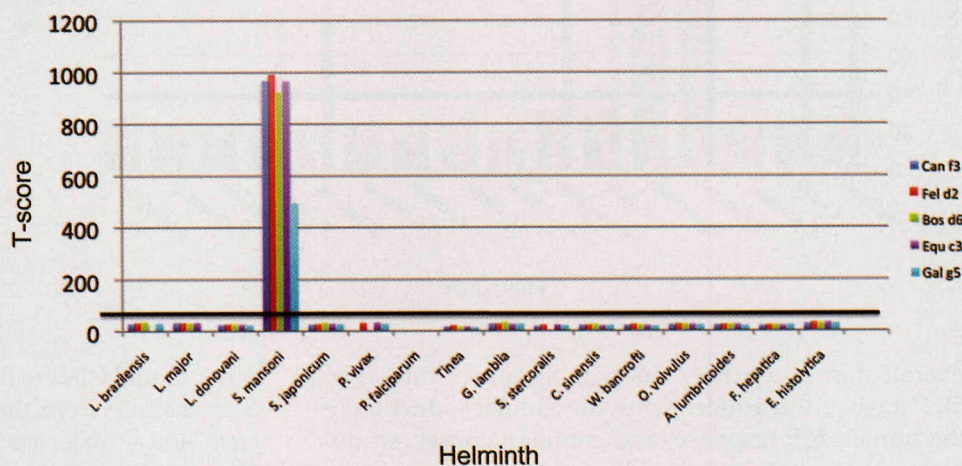


Figure 1. Significant homology between parasites and aeroallergen enolase. Significant homology of the enolase protein sequences found in fungi and plants compared with the enolase sequence in parasites. Parasites are on the x-axis and T scores are on the y-axis. Represented are the Fungi phyla Ascomycota (Alt a 6, Cla h 6, Cur l 2, Asp f 22, and Pen c 22), Basidiomycota (Rho m 1), the Plantae kingdom (Hev b 9), and those of 16 parasite sequences (Leishmania braziliensis, Leishmania major, Leishmania donovani, Schistosoma mansoni, Schistosoma japonicum, Plasmodium vivax, Plasmodium falciparum, Tinea, Giardia lamblia, Strongyloides stercoralis, Clonorchis sinensis, Wuchereria bancrofti, Onchocerca volvulus, Ascaris lumbricoides, Fasciola hepatica, and Entamoeba histolytica). The total computed value in all cases except for *S. stercoralis* and *Ascaris* is significant. The black bar represents a T score of 40, indicating a significant homology. (The E value was set at 10 for inclusion, with E values found <0.001 to be likely significant for homology. The T score was then recorded for each allergen–parasitic antigen pair. A greater T score indicates greater homology between the two sequences. T scores of <40 were associated with nonspecific E values and therefore nonhomologous sequences.)

Figure 2. Significant homology between parasites and aeroallergen albumin. T scores comparing the allergens representing albumin from the Chordates (Can f 3, Fel d 2, Bos d 6, Equ c 3, and Gal g 5) with the parasites are shown. Parasites are on the x-axis and T scores are on the y-axis. The T scores for Can f 3, Del d 2, Bos d 6, Equ c 3, and Gal g 5 all showed significant homology to the albumin in *Schistosoma mansoni*; however, no other parasites showed significant homology.



from mites, snails, nematodes, shrimp, and midges. None of the other parasites evaluated had any significant homology with any of the aeroallergens (Fig. 3).

Trypsin

The trypsin protein is represented by allergens all within the Astigmata order of the Arthropod class: Blo t 3, Der f 3, Der p 3, and Eur m 3. The allergen for Blo t 3, representing *Blomia tropicalis*, showed significant homology (T score, 138) with all parasites except *O. volvulus*, *Fasciola hepatica*, *S. stercoralis*, and *Entamoeba histolytica*. None of the other aeroallergens displayed a homologous trend in conserved domains (Fig. 4).

DISCUSSION

The IgE–eosinophil immune response originally developed as a consequence of the immune response of mammals to specific parasitic infections. Over the past decade, researchers have described an inverse relationship of atopic disorders and helminth infections with increasing evidence that helminth infections can protect the host against Th2-mediated allergic pathologies, even though helminthes themselves are strong Th2 inducers.^{9,12,24} In murine model systems, alleviation of allergy is not achieved through immune deviation to Th1 but is linked to expansion of regulatory T-cell activity.^{15,25} From these researchers' theories, we propose a schematic of the evolutionary process of IgE and

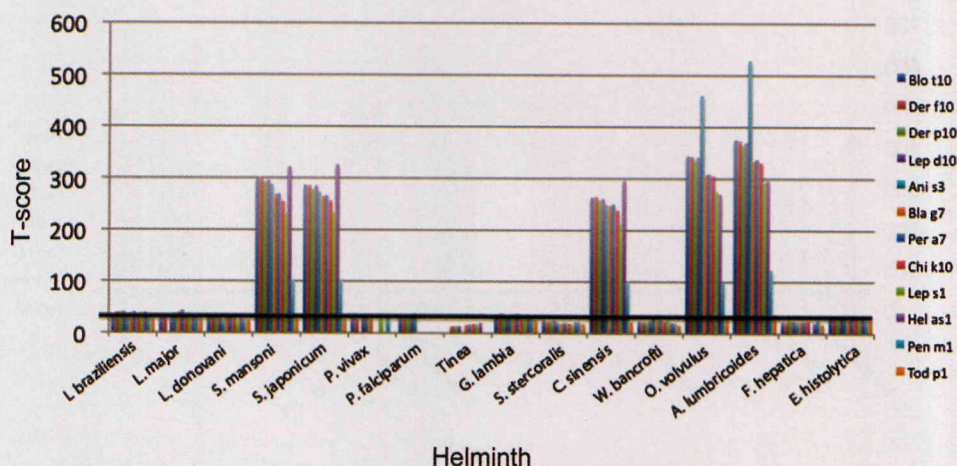


Figure 3. Significant homology between parasites and aeroallergen tropomyosin. Tropomyosin is represented by 12 separate allergens from across the arthropod phylum. Parasites are on the x-axis and T scores are on the y-axis. The *Tod p 1* allergen representing *Todarodes pacificus* (squid) showed no significant homology with any of the parasites. The other 11 allergens all showed significant homology (T scores of >100) for *Onchocerca volvulus*, *Schistosoma mansoni*, *Schistosoma japonicum*, and *Clonorchis sinensis*. None of the other parasites studied showed any significant homology.

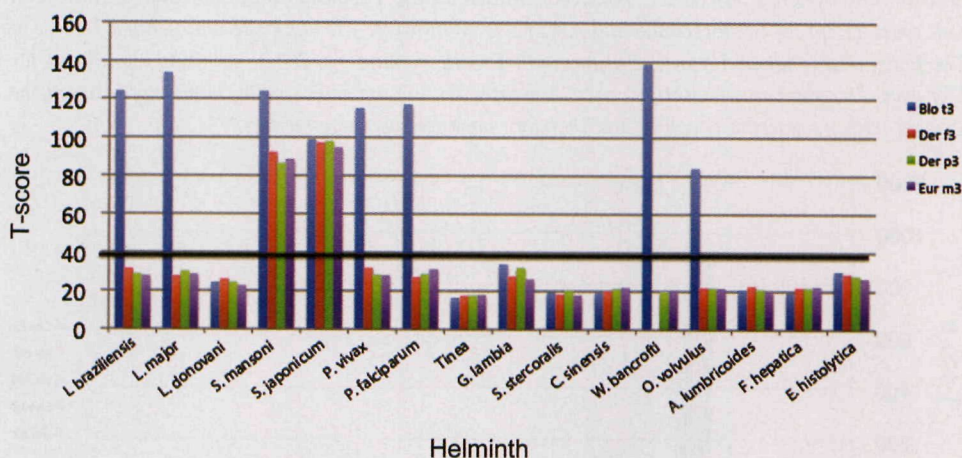


Figure 4. Significant homology between parasites and aeroallergen trypsin. The trypsin protein is represented by allergens all within the Astigmata order of the Arthropod phylum. Parasites are on the x-axis and T scores are on the y-axis. The trypsin sequence for *Blo t 3* showed significant homology with all parasites except *Clonorchis sinensis*, *Fasciola hepatica*, *Strongyloides stercoralis*, and *Entamoeba histolytica*. None of the other allergens showed significant homology.

overall shift in the Th1/Th2 paradigm over time (Fig. 5). Parasites and antigens provoke similar induction of the human IgE response, and common conserved domains that represent the allergenic epitopes can be observed. Metalloproteinase mimics the *S. japonicum* epitope as a potential vaccine, which is consistent with our data that tropomyosin showed strong homology between *S. japonicum* and *Blo t 3*, *Der f 3*, *Der p 3*, and *Eur m 3*.²⁶

The present results support the concept that common domains exist between species that stimulate the Th2 pathway response, including parasites and modern day aeroallergens. We found strong homologous protein sequences from known allergens and compared them against sequences from tropical and more temperate parasites. In a recent study, the induction of cross-reactive IgE antibodies to aeroallergens by helminthic infections suggested that the autoimmune response to parasites can induce allergic disease.²⁷ With epitope mapping studies, a three-dimensional com-

puter model was created for amino acids of glutathione S-transferase from the filarial parasite *Wuchereria bancrofti* and a major cockroach glutathione S-transferase allergen, *Bla g 5*, highlighting the conserved domains within the same epitope that bind human IgE (40% sequence identity).²⁷ In addition, tropomyosin from filarial parasites can induce IgE antibodies that cross-react with house-dust mite aeroallergens, and IgE responses to tropomyosins from *Ascaris* species cross-react with cockroach allergens,²⁸ consistent with our findings. Sensitization to common allergens, such as *Bla g 5* and tropomyosins, which are involved in human allergic disease, might have resulted from parasitic infections with cross-reactive protein domains.^{28–30}

The entire population of IgE epitopes for several aeroallergens is dependent on their structural integrity. The anaphylactic potential of any allergen may be decreased by disrupting the overall secondary structure of the protein. Epitopes might be composed of sequential residues along the polypeptide chain (continuous

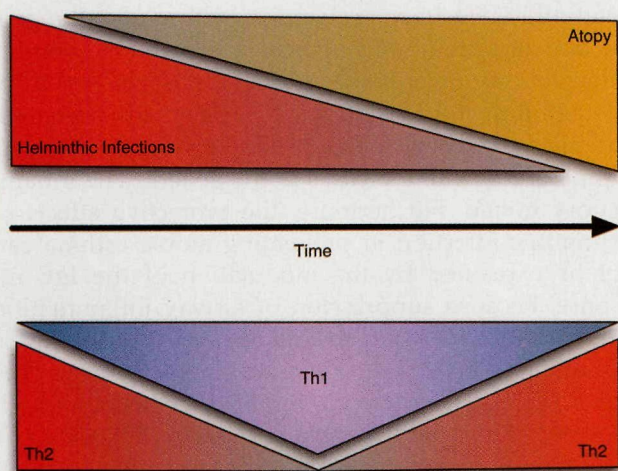


Figure 5. Conceptual time line of the evolution of immunoglobulin E (IgE). The balanced immune response between Th1 and Th2 pathways is heavily weighted to Th2 when infected with helminths. With time, the response shifts to Th1, corresponding to the migration of society from the nondeveloped parasitic-infected regions of the world to the more domesticated farmland and exposure to predominantly cell-mediated processes (tuberculosis, bacterial, and viral infection). With the advent of antimycobacterial, antibacterial, and antiviral treatments, there is a migration back to Th2 responses in the form of allergies.

or linear epitopes), as with most food allergens, or nonsequential residues from segments of the chain brought into physical proximity by the folded conformation of the protein (discontinuous or conformational epitopes). The majority of IgE epitopes are conformational, and a collapse in the structure can prevent allergen-specific IgE recognition and cross-linking. In this study, the aeroallergens primarily had conformational epitopes displayed on the surface of the protein, except for tropomyosin.

The enolase enzyme is a 46-kDa glycolytic enzyme that was highly conserved during evolution. It is located on the cell surface of eukaryotic cells, including yeast and parasites.³¹ In the present study, there was strong homology between the enolase of many parasites, fungi, and plants tested. The parasites in the *Leishmania*, *Schistosoma*, *Plasmodium*, *Giardia*, *Clonorchis*, *Wuchereria*, *Onchocerca*, *Fasciola*, and *Entamoeba* groups all had S scores of >200, and all but *Wuchereria* had an S score of >400. The only parasite that did not have an enolase with significant homology to the enolases of the allergen was *Ascaris*, possibly because of an incomplete enolase sequence in the NCBI database.

Enolase belongs to different phyla than tropomyosin, trypsin, and albumin, with Hev b 9 from the rubber tree serving as a prime example, showing just as strong homology to the parasitic antigens as the fungi. Hev b 9 showed the strongest homology with *Plasmodium falciparum* and *Fasciola* and equivalently strong homol-

ogy to the enolase of *Schistosoma*, *Plasmodium vivax*, *Clonorchis*, and *Onchocerca* than the other fungi enolases. Enolase allergens appear to be cross-reactive from different phyla, as indicated by the homology between the parasite enolase sequences and allergens derived from fungi and plants.

Albumin is an abundant protein and major contributor to colloid osmotic pressure of plasma in humans and other animals. Bovine serum albumin is useful in vaccine development against *S. mansoni*. When mimotopes of *S. mansoni* are conjugated to bovine serum albumin and the conjugates are used to immunize C57BL/6J mice, only the albumin-induced antibodies are effective at complement-mediated killing of *S. mansoni*.³² The present analysis of albumin-conserved domain data showed a cross-allergen, parasite-specific pattern in contrast with enolase, which had a cross-phyla, cross-allergen, parasite nonspecific pattern. We showed significant homology between albumin in *S. mansoni*, albumin represented by the five allergens tested, and albumins from across the Animalia phyla (Australian lungfish, zebra finch, and herring gull). *Plasmodium*, which has its entire genome mapped, had no homology to other allergens, including its albumin.

Although not typically seen with the albumin allergen, anticarbohydrate epitopes of invertebrate glycoproteins, from organisms such as *S. mansoni*, *Drosophila melanogaster*, and *Caenorhabditis elegans* (nematode worm), possess core α -(1,3)-fucosyltransferases, with synthesis of N-glycans that cross-react with anti-horseradish peroxidase antibodies.³³ The present results showed significant homology with the albumin of *M. musculus* and *S. mansoni*, but other workers have suggested that the observed homology may be caused by contamination and not horizontal gene transfer.³⁴

Tropomyosin is a well-known panallergen that belongs to a family of highly conserved proteins found in muscle and nonmuscle cells of invertebrates. Allergenic invertebrates include crustaceans (shrimp, crab, and lobster), arachnids (dust mites), insects (cockroaches and midges), and mollusks (squid, snail, and oyster), but vertebrate tropomyosins are nonallergenic. Increased exposure to mite antigens *via* immunotherapy may cause sensitization to cross-reactive seafood tropomyosins.³⁵ However, these studies neglect to compare duration of exposure. Although *in vitro* cross-reactivity among tropomyosins exists in different invertebrate species, it is unknown whether acute or chronic exposure and sensitization to a particular food allergen may cause sensitization to certain aeroallergens.

Tropomyosin also may be an important antigen that induces the IgE-eosinophil response in nematode infections. The present data showed significant homology in only a select few parasites (*O. volvulus*, *S. mansoni*, *S. japonicum*, *C. sinensis*, and *A. lumbricoides*). *O.*

volvulus and *A. lumbricoides* both had strong T scores with *Anisakis simplex* (Ani s 3), which is consistent with other studies, most likely because nematode allergens include paramyosin from *A. simplex*.^{36–38} Cross-reactivity exists between *B. tropicalis* (an important domestic dust mite in the tropics and subtropics) and *O. volvulus*, *S. mansoni*, *S. japonicum*, *C. sinensis*, and *A. lumbricoides* but not other tropical parasites. The reasons for selectivity of cross-reactivity toward distinct parasites are unknown. *S. japonicum* has a protective effect on gastrointestinal infections, further supporting the concept that cross-reactivity exists and that helminths play a pivotal role in balancing the Th1 and Th2 pathways.^{38,39}

Trypsin is a serine protease, a digestive enzyme of the gastrointestinal system, and an important antigen that mediates allergies to various arthropods, especially Blo t 3, the house-dust mite. The present results showed that Blo t 3 uniquely displays strong homology with all other allergens except *F. hepatica*, *C. sinensis*, *E. histolytica*, and *S. stercoralis*. Sequence alignment showed that the serine protease active sites are highly conserved among the clinically important mite species, including *B. tropicalis*.⁴⁰ All dust mites (Blo t 3, Der f 3, Der p 3, and Eur m 3) exhibited significant homology to both *Schistosoma* groups (*S. mansoni* and *S. japonicum*) but to none of the other species.

In this study, we investigated parasites and aeroallergens that bind to similar antigens (enolase, albumin, trypsin, and tropomyosin) and have shown homologous domains across different species (Table 2). Lectin-dependent conserved glycan antigens have significant homologies with filarial (*Brugia malayi*, *O. volvulus*, and *W. bancrofti*) and canine aeroallergens, which may contribute to Th2 adaptive responses.⁴¹ At a molecular level, these data are consistent with previous studies that documented an inverse relation between parasitic disorders that perturb the mucosal integrity of the gastrointestinal tract and allergies that reflect a perturbation of mucosal immunity in other mucosal regions of the body.⁴² Thus, helminthic colonization may provide protection against or diminution of the allergic and asthmatic response, suggesting a potential role for shared antigens between parasites and environmental aeroallergens in modulating the immune response.^{13,15,20,43,45} Previous studies noted that IgE levels and eosinophil counts decreased with antiparasitic treatment, but when atopic disorders such as asthma were treated with the same infectious agents (hookworms and whipworms), the IgE response was redirected.^{46–48}

The role of infections in preventing atopy and asthma has gained much support in recent years with the hygiene hypothesis. The hygiene hypothesis is based on epidemiological studies that suggest that early exposure to infections may prevent the development of atopy.^{49,50} The immunologic mechanisms responsible for IgE production that are protective in

helminthic infections are similar to those for the production of specific IgE against allergens. Helminthic colonization prevents the subsequent development of allergen-induced eosinophilic airway inflammation and allergen-specific Th2 cytokine production despite promoting overall increases in the general Th2 inflammatory milieu. Furthermore, the protective effects of helminthic infection in preventing atopic asthma can not be explained by the modulation of the IgE response, because suppression of airway inflammation by helminthic infection occurs despite increased levels of antigen-specific IgE. Helminths may decrease the risk for allergies by stimulating the production of high levels of polyclonal IgE that are capable of blocking Fc receptors on mast cells or by promoting high levels of regulatory cytokines capable of down-regulating allergic responses.⁶

The association between the memory T-cell subpopulation D4⁺CD45RO⁺ and the stimulation of IgE against both parasites and allergens is dependent on the regulatory cytokine interleukin 10 (IL-10).^{15,20} These data, which parallel changes in IL-5 release, are most consistent with the explanation that helminth-infected mice have reduced allergen-specific Th2 responses. The induction of the regulatory cascade, characterized by IL-10 releasing T cells, has been linked to immune modulation by helminths in several disease models, including allergic²⁵ and autoimmune diseases.^{51–53} The suppression of IL-10 release in helminth-infected mice may represent reductions in antigen responsive Th2 cells. Indeed, the induction of IL-10 by mitogen stimulation could reflect either a larger non-antigen-specific Th2 population, or alternatively, a release of cytokine from active regulatory cells. T cells with non-antigen-specific regulatory properties may be induced. These include T-regulatory cells that may release both IL-10 and IL-5 but its effects are IL-10 dependent.^{54,55}

In summary, the present data indicated that conserved domains exist in specific parasites and allergens (Table 2). This supports the theory that the human IgE–eosinophil immune response to common allergens could be a direct consequence of constant stimulation of parasitic organisms. The data showing homologies between parasites and allergens add important information and support observations and studies regarding the protective effect of parasitic infections on the development of atopy and asthma.^{21,56–62} This may explain how the human IgE response to the selected helminthic enzymes are responsible for allergy to many common aeroallergens because of homologous conserved domains. Although amino acid sequence homology does not predict antigen cross-reactivity with IgE antibodies, additional studies may better help explain the immune response relation between aeroallergens and parasitic antigens and whether molecular mimicry is responsible. With further research studying the evolution of the host–parasite relation and im-

immune response and the increasing availability of helminth genome data, it may be possible to identify many more conserved domains between aeroallergens and helminths. This may facilitate the development of methods for targeted immunotherapy.

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