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Structural and immunologic crossreactivity among filarial and mite tropomyosin: implications for the hygiene hypothesis

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Abstract

Background—The "Hygiene Hypothesis" suggests that parasitic infection modulates host immune responses and decreases atopy. Other data suggest parasitic infections may induce allergic responsiveness.

Objective—Assess the structural and immunologic relationships between the known *Dermatophagoides pteronyssinus* (Der p 10) tropomyosin allergen and filarial tropomyosin (OvTrop).

Methods—The molecular, structural, and immunologic relationships between OvTrop and Der p 10 were compared. Levels of OvTrop- and Der p 10-specific IgE, IgG, and IgG4 in sera of filaria-infected and -uninfected *D. pteronyssinus*-atopic individuals were compared as were the responses in nonhuman primates infected with the filarial parasite *Loa loa*. Crossreactivity was compared by antigen-mediated depletion assays and functionality by passive basophil sensitization.

Results—Filarial and mite tropomyosins were very similar, with 72% identity at the amino acid level and overlapping predicted 3D structures. The prevalence of IgE and IgG to Der p 10 was increased in filaria-infected individuals compared with uninfected subjects. There was a strong correlation between serum levels of Ov- and Der p 10-tropomyosin-specific IgE, IgG, and IgG4 ($P < 0.0001$; $r > 0.79$). Pre-incubation of sera from anti-Der p 10 positive subjects with OvTrop completely depleted IgE, IgG, and IgG4 anti-Der p 10. Basophils sensitized with sera from individuals allergic to Der p 10 released histamine similarly when triggered with OvTrop or Der p 10. Primates experimentally infected with *L. loa* developed IgE that crossreacted with Der p 10.

Conclusion—Filarial infection induces strong crossreactive antitropomyosin antibody responses that may affect sensitization and regulation of allergic reactivity.

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Keywords

Allergy; antibody crossreactivity; *Dermatophagoides pteronyssinus*; filariasis; hygiene hypothesis; tropomyosin

Allergies are inflammatory disorders in which the complex interplay among environmental, nutritional, and genetic factors has been implicated in underlying their development.^{1, 2} It is clear that the incidence of allergic diseases is increasing, not only in resource-rich countries of the world but also in the urban areas of resource-poor countries.³ An appealing explanation for such increase in incidence is the so-called "hygiene hypothesis," which suggests that lack of exposure by children early in their development to microbes or their components (as occurs with urbanization and widespread antibiotic use) may explain the increased incidence of allergic diseases.^{4, 5} The hygiene hypothesis is particularly intriguing in the context of human helminth infections, in that helminth-infected individuals have typically been found to have lower prevalences of atopy^{6–8} than helminth-uninfected subjects and where autoimmune diseases have been found to be modulated by concomitant helminth infection^{9, 10} or helminth-derived products.¹¹ In contrast, other studies have suggested that the presence of intestinal worms is associated with an increased incidence of atopy^{12, 13} or asthma. For example, a meta-analysis of 30 clinical studies concluded that hookworm infection protects against asthma but that *Ascaris lumbricoides* tended to aggravate clinical asthma symptoms,¹⁴ while other intestinal parasites (e.g., *Trichuris trichiura*, *Enterobius vermicularis*, and *Strongyloides stercoralis*) had no effect on development of asthma.¹⁴ Clearly, the effect of helminth infections on asthma may also be influenced by the nature of the bronchial hyperreactivity itself (i.e., allergic or non-allergic). Accordingly, a recent study found that *A. lumbricoides* infection decreases atopic sensitization but increases exercise-induced bronchoconstriction.¹⁵ Nonetheless, the effect of worm parasites on allergic diseases appears to be dependent on how an individual parasite species interfaces with the host immune response as well as other factors including the length of infection (chronicity).

The mechanisms involved in the increase of prevalence of asthma among patients infected with *A. lumbricoides* are still a matter of debate, although crossreactivity between helminth proteins and specific allergens is thought to play a role.¹⁶ Crossreactivity of B cell epitopes among allergens is an important factor in allergic cross-sensitization and is the apparent cause of the oral allergy syndrome.¹⁷ For instance, between 38% and 99% of patients allergic to Bet v 1 (a major birch pollen allergen) develop hypersensitivity to certain foods as a consequence of the antigenic crossreactivity of Bet v 1 with its homolog in apples (Mal d 1), celery (Api g 1), and other plant foods.

Another example is the crossreactivity among tropomyosins of different species.¹⁸ Tropomyosins are highly conserved among different species, making crossreactivity a likely possibility. Interestingly, tropomyosins of nonhuman vertebrates are not immunogenic in humans and do not cause allergy.¹⁹ In contrast, tropomyosins from invertebrates—the major allergens of seafood that typically have identities (at the molecular level) of less than 55% with human tropomyosins—are strong inducers of IgE in humans.¹⁹ For example, allergic orthodox Jews (never exposed orally to shrimp or other crustaceans) were found to have IgE anti-shrimp tropomyosin (Pen a 1), felt to be a result of cross sensitization with tropomyosin of the house dust mite (HDM) (Der p 10) or cockroach (Bla g 7).²⁰ Furthermore, some studies have reported crossreactivity between tropomyosin of *A. lumbricoides* and the tropomyosins of the cockroach allergen Bla g 7^{16, 21} or the storage mite *Blomia tropicalis* (Blo t 10).²¹

We investigated the relationship between filarial tropomyosin (OvTrop) and the tropomyosin of *Dermatophagoides pteronyssinus* (Der p 10). Filarial infections are particularly informative in studying the helminth-allergy interface, because they are tissue-invasive, systemic infections that induce not only high levels of parasite-specific and polyclonal IgE and IgG4 but also, because of their chronicity, high levels of IL-10 that modulate T cell (and perhaps B cell) responses.^{22, 23}

Thus, the present study demonstrates marked similarities at the amino acid and structural level between filarial tropomyosin and Der p 10. More impressive, however, is the marked crossreactivity of tropomyosin-specific IgE and IgG (and IgG4). Moreover, using sera from experimentally filaria-infected nonhuman primates, we could demonstrate unequivocally the development of antifilarial tropomyosin IgE that was entirely crossreactive with Der p 10. Such strong crossreactivity for both IgE and IgG provides clear insights into the relationship between allergic disease and concomitant helminth infection.

METHODS

Patients and sera

Sera from well characterized filaria-infected (Fil⁺) individuals were utilized in this study.²⁴ All patients were seen by the Clinical Parasitology Unit 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 53 individuals infected with *Loa loa*, 11 infected with *Onchocerca volvulus*, and 4 infected with *Wuchereria bancrofti*. Sera from 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). Those showing levels below 0.35 kUA/l were considered negative and categorized as non-atopic. Sera from the Phadiatop®-positive subjects were further tested for house dust mite-specific IgE using an Immucap assay specific for Der p (Phadia). Individuals positive for HDM (levels above 0.35 kUA/l) were considered HDM atopic. Based on these data, the 126 subjects were divided into four groups based on their HDM allergy and filarial infection status: 1) Filaria⁻ and non-atopic, Ni-NA; *n* = 21 individuals; 2) Filaria⁻ and atopic, Ni-A; *n* = 37; 3) Fil⁺ and non-atopic, Fil-NA; *n* = 19; and 4) Fil⁺ and atopic, Fil-A; *n* = 49.

Antigens

cDNA encoding tropomyosin of *D. pteronyssinus* (Der p 10) or *O. volvulus* (OvTrop) was cloned into bacmids (termed pB3930-X1-603 and 3930-X2-603, respectively). Transformed baculoviruses were used to infect Hi5 cells for expression of GST-fusion proteins. Cell lysates and supernatants were purified through the GST tag that was later removed by digestion with TEV protease followed by dialysis. The purity and integrity of OvTrop and Der p 10 were assessed by SDS-PAGE. Other antigens used were crude extracts from *Brugia malayi* adult worms, termed BMA,²⁵ and timothy extract (ALK Abello, Port Washington, New York).

ELISA for Der p 10 and OvTrop

Measurements of Der p 10 and OvTrop antibodies were performed by enzyme-linked immunosorbent assay (ELISA). Flat-bottom plates (Immulon 4; Dynatech, Chantilly, Virginia) were coated overnight at 4°C with 1 µg/mL of antigen (Der p 10 or OvTrop) in PBS followed by washing with PBS and 0.05% Tween (Sigma Chemical, St. Louis,

Missouri). Plates were then blocked with PBS/BSA 1% for 1 h at room temperature. Serum samples or a standard curve made of positive sera were diluted in PBS/BSA 1% and incubated overnight at 4°C. Plates were then washed and incubated with polyclonal goat anti-human IgE (R&D, Minneapolis, Minnesota), monoclonal mouse anti-human IgG4 (Hybridoma Reagent Laboratories, Baltimore, Maryland) or alkaline phosphatase-conjugated anti-human IgG (Jackson ImmunoResearch, West Grove, Pennsylvania) for 1 h at room temperature. After washing, plates were incubated with alkaline phosphatase-conjugated anti-goat IgG or anti-mouse IgG for the IgE and IgG4 plates for 1 h at room temperature. Plates were again washed, and p-nitrophenylphosphate disodium (Sigma Chemical) was added at 1 mg/ml in sodium carbonate buffer (KD Medical, Columbia, Maryland). Colorimetric development was detected at 405 nm using a microplate reader (Molecular Devices, Sunnyvale, California) and OD levels interpolated in the standard curve. Geometric mean (GM) + 3 SD of the antibody levels of the NA-Ni group were used to set cut-off values for Der p 10 and OvTrop ELISA to define individuals positive and negative for antibodies anti-Der p 10 and anti-OvTrop.

Depletion ELISA—Flat-bottom plates (Immulon 4; Dynatech) were coated overnight with 10 µg/mL of Der p 10, OvTrop, or nonrelated recombinant OvGST antigen, then washed, and blocked as described above. Sera were added to the plates in various dilutions and incubated overnight. On the following day, sera were transferred to plates coated overnight with Der p 10 (1 µg/mL) after appropriate washing and blocking, and ELISA were performed as described above.

Primate sera

Sera from rhesus monkeys and baboons experimentally infected with *L. loa*²⁶ and their respective controls were used to examine development of the antibody response to tropomyosins. Only animals that had sera from both pre-infection and at 12 months following infection were utilized. All sera had been cryopreserved at -80°C until used. ELISA for monkeys were performed as for humans.

Passive basophil sensitization and histamine release

Passive basophil sensitization was performed as described previously.²⁴ Basophils were stimulated with increasing concentration of antigens or anti-IgE to assure appropriate IgE stripping and IgE binding after sensitization for 30 min at 37°C. Histamine was measured in the supernatant with a commercial EIA (Beckman Coulter, Marseilles, France) used according to the manufacturer's instructions.

Bioinformatic analysis

The sequence of OvTrop (Acc. No Q25632.1) was used to compare the protein sequences from other helminths, crustaceans, mites, and cockroaches using blastp.^{27, 28} Multiple sequence alignments of the tropomyosins of *Acanthocheilonema vitae* (Av) (O01673.1), *Ascaris lumbricoides* (Al) (ACN32322.1), *Anisakis simplex* (Ani s 3) (Q9NAS5.1), *Brugia malayi* (Bm) (translated from partial expressed sequence tag, AA585557.1), Der p 10 (O18416.1), *Dermatophagoides farinae* (Der f 10) (Q23939.2), *Lepidoglyphus destructor* (Lep d 10) (Q9NFZ4.1), *Blattella germanica* (Bla g 7) (Q9NG56.1), *Periplaneta americana* (Per a 7) (Q8T6L5.1), *Charybdis feriatus* (Cha f 1) (Q9N2R3.1), *Homarus americanus* (Hom a 1) (O44119.1), *Metapenaeus ensis* (Met e 1) (Q25456.1), *Panulirus stimpsoni* (Pan s 1) (O61379.1) and *Farfantepenaeus aztecus* (Pen a 1)(AAZ76743.1) were performed using ClustalW, and distance trees were obtained using Lasergene MegAlign (DNASTar, Inc., Madison, Wisconsin). 3-D-structural predictions were carried out using homology modeling "ab initio" from Rosetta (<http://www.bioinfo.rpi.edu/~bystrc/hmmstr/server.php>) and

Swissmodel (<http://swissmodel.expasy.org>) that used *Sus scrofa* tropomyosin (1c1gA) as a model on which to base the Der p 10 (identity of 56.9%; e-value of 8×10^{-30}) and OvTrop (identity of 56.9%; e-value of 10^{-29}) structures.

Statistical analysis

GraphPad Prism v5.0 (GraphPad Software Inc., San Diego, California) was used to prepare graphs and perform statistical analyses. Comparison of antibody prevalence was performed using a one-tailed Fisher's exact test. Relative risks (RR) and odds ratios (OR) with their respective approximate confidence intervals (CI) were calculated in GraphPad Prism. Correlations were performed using Spearman rank correlation analysis.

RESULTS

Tropomyosin is a well conserved protein among different species and different phyla. Bioinformatic analysis (Fig 1) showed extremely close alignments of filarial tropomyosins from *O. volvulus*, *A. vitae*, and *B. malayi* (partial sequence based on expressed sequence tags) with tropomyosins from other helminths (e.g., *A. lumbricoides*, *A. simplex*) and those from mites, cockroaches, and crustaceans. Homology at the amino acid level among the different tropomyosins in which full-length sequence was available varied from 67–98% in identity (e-values ranging from 10^{-97} to 10^{-167}). The conserved N-terminal DAIKK (Fig 1, A) and the tropomyosin signature LKEAExRAE were also present in all tropomyosins analyzed (Fig 1, A). Filarial tropomyosins also clustered with those tropomyosins known to be allergenic (Ani s 3, Der p 10, Bla g 7, Pen a 1, and others) when phylogenetic trees were constructed (Fig 1, B). Of note, the identity between OvTrop and Der p 10 was 72%, (with a similarity of 87% and an e-value of 3×10^{-167}), thereby showing a high level of sequence conservation. Moreover, 3-D modeling predictions of Der p 10 and OvTrop were found to be indistinguishable (Fig 1, C) with both proteins with length sizes of 414.2 Angstroms.

To address the crossreactivity between filarial tropomyosin and HDM tropomyosin, sera from Fil⁺ and Fil⁻ individuals were defined as being from atopic or non-atopic donors based on a positive response to a pool of common allergens (Phadiatop®). Positive individuals were screened further for specific reactivity to *D. pteronyssinus*, and four groups were defined with respect to HDM allergy: 1) Filaria⁻, non-Atopic or Ni-NA; 2) Filaria⁻, Atopic or Ni-A; 3) Filaria-infected, Non-Atopic or Fil-NA; and 4) Filaria-infected, Atopic or Fil-A.

Levels of Der p 10-specific and OvTrop-specific IgE, IgG, and IgG4 were measured (Fig 2; Table 1). The prevalence of IgE and IgG antibody to Der p 10 was increased in the Fil-A group when compared with Ni-A, with 16/49 Fil-A having antigen-specific IgE antibodies and 17/47 having antigen-specific IgG compared with 3/37 in the Ni-A group for IgE and 2/37 for IgG ($P < 0.004$ for both comparisons). When the RR and OR were calculated comparing the two HDM atopic groups (Ni-A and Fil-A), both analyses showed that Fil⁺ individuals had significantly increased likelihoods of having increased levels of Der p 10-specific IgE and IgG antibodies (Table I). Although IgG4 anti-Der p 10 antibodies showed a similar trend in the Fil⁺ group, the RR/OR did not reach statistical significance. Antibody levels to OvTrop had the same pattern seen for antibody levels against Der p 10 (Fig 2).

When the antibody levels obtained for Der p 10 and OvTrop by ELISA were compared, a strong relationship between levels of the IgE, IgG, and IgG4 anti-OvTrop antibodies and the respective isotypes of anti-Der p 10 (with $r > 0.79$ and $P < 0.0001$ for each antibody class tested; Fig 3, A) was seen. To test the crossreactivity between the two tropomyosins, depletion ELISA was performed (Fig 3, B). As shown, pre-incubation of Der p 10 positive sera with OvTrop was able to deplete Der p 10-specific IgE, IgG, and IgG4 to levels comparable to sera pre-incubated with Der p 10. Pre-incubation with a recombinant

nonhomologous protein (OvGST, used as negative control) showed no depletion of Der p 10-specific antibodies. In addition, sera from mice vaccinated with Der p 10 developed antibodies that not only recognized native tropomyosin in mite extract but also could be inhibited by Der p 10, Ovtrop, mite extract, and crude parasite extract (data not shown). To investigate the functionality of the crossreactive antibody further, we used sera of three individuals (two Fil-A and one Ni-A) positive for anti-tropomyosin (Der p 10 or OvTrop) to passively sensitize basophils. Basophils sensitized with IgE from these three individuals responded indistinguishably to increasing amounts of either Der p 10 or OvTrop antigens by releasing histamine (Fig 4).

To demonstrate unequivocally that crossreactive antitropomyosin antibodies are induced by filarial infection, sera of nonhuman primates experimentally-infected with *L. loa*, one of the few human filarial pathogens that have a nonhuman permissive host, were analyzed for Der p 10-specific IgE. As expected, Fil⁺ animals developed IgE specific to filarial antigens (BMA) (Fig 5), while the control animals did not. In the first month following infection, each monkey infected with *L. loa* displayed increased levels of IgE anti-Der p 10, with an approximate increase of 50% over pre-infection levels. The peak, however, was reached at the latest time points evaluated, 24–30 months post infection, with increases over 5 times above the pre-infection levels being observed. Not surprisingly, the responses to Der p 10 were paralleled by those to OvTrop. Interestingly, the kinetics of development of the IgE anti-OvTrop or Der p 10 responses were somewhat different from those to BMA. To exclude the possibility of the IgE anti-tropomyosin being a result of polyclonal IgE activation of B cells (as has been postulated based on early animal models),^{29–31} IgE to timothy was assessed in the sera of infected and uninfected monkeys. Neither the Fil⁺ nor the control group altered their anti-timothy IgE levels from background levels over the entire course of the infection (Fig 5).

DISCUSSION

Tropomyosins are not only major allergens of seafood, mites, and cockroaches but also are highly immunogenic helminth proteins.¹⁸ Helminth-infected patients have shown strong antitropomyosin antibody responses,³² and filarial tropomyosins have been found to confer partial immunity in nonpermissive, vaccinated animals.³³ Because of their level of sequence conservation, the crossreactivity among tropomyosins has been predicted and studied previously in the context of the filarial parasite *O. volvulus*.^{18, 32} In the present study, we have not only confirmed the immunogenicity of tropomyosin in filarial infection but, more importantly, have demonstrated that the antitropomyosin antibodies induced in filarial infection are crossreactive with those allergenic tropomyosins of invertebrates.

Induction of crossreactive HDM-specific IgE induced by filarial infection lends credence to the concept that immune responses to tropomyosin could account for the increased prevalence of atopy and asthma seen in some helminth-infected populations, particularly those with the intestinal parasite *A. lumbricoides*.¹⁶ Although high levels of IgE anti-mite tropomyosin could mediate allergic responses, it is likely that regulatory mechanisms such as IL10 and Tregs, induced by chronic helminth infection may alter allergic reactivity³⁴. Moreover, as there are concomitant increases in IgG antitropomyosin antibodies, these could provide an additional counterbalancing mechanism by acting as a "blocking" antibody as has been shown previously.^{35, 36}

IgG can regulate allergic responsiveness by two main mechanisms: epitope competition and signaling through FcγRIIB. Epitope competition, one of the first mechanisms considered to regulate allergic responses,³⁷ is a concept that postulates that IgG that bind to the same epitopes as the IgE have the ability to block the access of allergens to basophil- and/or mast

cell-bound IgE, thereby preventing the development of immediate-type hypersensitivity reactions. This mechanism may also underlie some other recently suggested pathways by which IgG can modulate allergic reactivity by diminishing IgE-facilitated antigen presentation to T cells^{38, 39} or by directly downregulating IgE production^{40, 41} by B cells.

A more recently described mechanism involves FcγRIIB (CD32B), an immunoreceptor tyrosine-based inhibition motif-associated receptor that has been shown to modulate the activation of dendritic cells, B cells, and T cells.^{42, 43} This receptor has been shown to modulate IgE-mediated activation by mast cells and basophils,^{44, 45} even under conditions in which IgE is being crosslinked. This has led to a new vaccination strategy for allergic diseases such that either the CHε and a CHγ are fused through a hinge region⁴⁶ or a recombinant allergen is fused with CHγ to induce a negative signal, thereby forcing the inhibitory FcγRIIB to associate with FcεR and constraining cell activation.⁴⁷

Because helminth parasites, and especially filariae, are known to drive strong IgG4 responses,⁴⁸ the fact that we were able to demonstrate crossreactivity between common mite and helminth tropomyosin for both IgG (dominated by IgG1 isotypes) and IgG4 is not surprising. Nevertheless, if helminth infection induces a Th2-rich environment, there is a likelihood that crossreactive IgE antibodies will predominate; however, because of their chronic nature, over time these infections will induce a modified (or regulated) T cell environment^{22, 49} that will be dominated by IgG4 and IL-10 and that could, in turn, regulate allergic phenomena. Thus, alteration of the balance between IgE-promoting and IgE-regulating environments may provide the missing link in understanding not only the interplay between helminth infection and allergic diseases, but may also provide new insights into tools for intervention in allergic diseases.

Key Message

- There is strong IgE and IgG antibody crossreactivity between filarial tropomyosin and house dust mite tropomyosin (Der p 10) that may account for the high prevalence of allergen-specific IgE seen among filaria-infected individuals
- The helminth infection by itself is sufficient to induce crossreactive IgE antibodies and cause allergic sensitization
- The role of parasite-induced crossreactive IgG in regulating parasite-induced cross-reactive IgE sensitization may be a mechanism to limit IgE-mediated effector responses

Abbreviations used

BMA	crude extract from <i>Brugia malayi</i> adult worms
CI	confidence interval
Der p	<i>Dermatophagoides pteronyssinus</i>
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
HDM	house dust mite
Ni-A	Fil ⁺ and atopic
Ni-NA	Fil ⁺ and non-atopic
OR	odds ratio
OvTrop	tropomyosin of <i>Onchocerca volvulus</i>
PBMC	peripheral blood mononuclear cell
RR	relative risk

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A **B** **C** **FIG 1.**

A, Protein sequences of tropomyosin of helminths, mites, cockroaches, and crustaceans were aligned. Identical amino acids have one-letter codes and are black. Conserved amino acid groups are gray; nonconserved amino acids are non-shaded dots. Red letters show conserved N-terminus and tropomyosin signature sequences. **B**, Phylogenetic tree showing distances among tropomyosin family groups. **C**, 3-D structure predicted for OvTrop and Der p 10 with Rosetta and Swissmodel.

FIG 2.

Increased prevalence of anti-Der p 10 antibodies in Fil+ subjects. Anti-OvTrop and -Der p 10 antibodies were measured in Ni-NA (Fil⁻ non-atopic), Ni-A (Fil⁻ atopic), Fil-NA (Fil⁺ non-atopic), and Fil-A (Fil⁺ atopic). Dots, individual OD for IgE and IgG4 (serum dilution 1:25) and IgG (1:400). Dotted lines, GM + 3 SD of normal controls for each ELISA (set as cut-off values for assay).

A **B** **FIG 3.**

A, Correlation between anti-OvTrop (y-axis) and anti-Der p 10 (x-axis) IgE (left panel), IgG (middle panel), and IgG4 (right panel); each point represents an individual. **B**, Results of depletion by Der p 10 (closed circles), OvTrop (open circles), or control antigen (closed triangles) on IgE (left panel), IgG (middle panel), and IgG4 (right panel) responses to Der p 10 as a function of serum dilution.

FIG 4.

Der p 10 and OvTrop are functionally indistinguishable for eliciting IgE-mediated responses. Basophils sensitized with sera of atopic and Fil⁺ (#1 and #2) or atopic but Fil⁻ (#3) individuals were stimulated with increasing concentrations of Der p 10 or OvTrop. Insert: Donor basophils activated with anti-human IgE before and after IgE stripping and following IgE sensitization. Histamine release as function of antigen concentration is expressed as the percentage of maximum histamine content.

FIG 5.

Crossreactive IgE develops in nonhuman primates infected with *Loa loa*. Infected monkeys ($n = 4$) were bled before infection and at various time points after infection; uninfected control monkeys ($n = 3$) were bled at the same time points. Left panel, pre-infection levels (dots represent each animal); right panel, percentage increase in specific IgE levels over background (bars represent GM + standard error).

TABLE I

Prevalence of IgE and IgG to tropomyosin are increased in filaria-infected populations

	Prevalence (%) [*]			RR (CI)	Odds Ratio (CI)	<i>P</i> value [†]
	Ni-A (<i>n</i> = 37)	Fil-A (<i>n</i> = 49)				
IgE	8.1	32.7	3.2 (1.10–9.34)	5.4 (1.46–20.60)	0.0060	
IgG	5.4	36.2	5.1 (1.35–19.30)	9.9 (2.11–46.40)	0.0005	
IgG4	13.5	30.6	1.4 (1.03–2.05)	2.8 (0.91–8.66)	0.0529	

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

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