

IgE memory: Persistence of antigen-specific IgE responses years after treatment of human filarial infections

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Background: The human immune response to helminth infections is characterized by elevated serum levels of antigen-specific IgE. Although it is well established that IgG humoral immunity can persist for years, as yet there have been no longitudinal studies of antigen-specific IgE responses to helminth infection in the absence of re-exposure to pathogen.

Objective: We sought to determine the presence of filaria-specific IgE responses years after treatment of human filarial infections in patients no longer living in a region endemic for filariasis.

Methods: Filaria-specific IgE levels were measured in 34 filaria-infected patients before and 11 to 178 (median = 33) months after anthelmintic therapy. Frequencies of filaria-specific IgE-producing cells and measures of filaria-specific IgE-mediated histamine and IL-4 release from basophils were measured in subsets of these patients.

Results: At follow-up, all patients were asymptomatic without evidence of active infection, and none had traveled to filaria-endemic regions since initial evaluation. Although frequencies of *Brugia malayi* antigen (BmA_g)-specific IgE-producing cells and serum levels of BmA_g-specific IgE decreased significantly over time, both remained detectable in the majority of patients years after initial treatment. Six of 10 patients' basophils released histamine in response to BmA_g after treatment (vs 7 of 10 before treatment). Basophils also continued to release IL-4 after stimulation with BmA_g years after treatment.

Conclusion: These results demonstrate that filaria-specific IgE production and filaria-specific IgE-mediated basophil release of histamine and IL-4 persist for years after treatment of human filarial infections.

Clinical implications: These findings suggest that (1) absolute allergen avoidance may not result in the loss of allergy, and (2) parasite-specific IgE-inducing vaccines, if effective, could potentially induce longstanding protection. (*J Allergy Clin Immunol* 2006;117:939-45.)

Key words: IgE, immunologic memory, histamine, IL-4, helminth, basophil, humoral immunity, filaria, parasite

Abbreviations used

BmA_g: *Brugia malayi* antigen

GM: Geometric mean

NIAID: National Institute of Allergy and Infectious Diseases

Although it is well established that humoral immunity can persist and be protective for decades even in the absence of re-exposure to a pathogen, the classic studies that have demonstrated this have focused on pathogens that induce antigen-specific IgG responses.¹⁻³

In contrast to bacterial, viral, and protozoan infections, the human immune response to helminths is characterized by elevated serum levels of antigen-specific and polyclonal IgE.⁴⁻⁶ Indeed, antigen-specific IgE has been shown to be an important component of protective immunity in multiple animal models of helminth infection⁷⁻⁹ and to be associated with decreased rates of helminth infection in human beings.¹⁰⁻¹²

Although several studies have examined the duration of antigen-specific IgE responses in allergy,¹³⁻¹⁶ there have been only 2 longitudinal studies quantifying parasite-specific IgE levels after helminth infection. One, performed on a cohort of 17 patients with trichinellosis in Mexico, examined patients at 1 year after initial infection,¹⁷ and the other, performed on 27 patients with onchocerciasis in Guatemala, evaluated patients at 2 years after treatment with ivermectin.¹⁸ In both studies, all patients tested had helminth-specific IgE at follow-up. A limitation of both of these studies was that re-exposure could not be excluded because patients had remained in areas of endemicity in both studies.

The goal of this study was to evaluate the persistence of antigen-specific IgE levels and antigen-specific IgE-mediated activation of basophils years after treatment of filarial infection in human beings. Filarial parasites are arthropod-borne tissue-dwelling helminths that are a major worldwide burden of disease. Pathogenic human filariae include *Wuchereria bancrofti* and *Brugia malayi*, which cause lymphatic filariasis; *Onchocerca volvulus*, the cause of onchocerciasis (river blindness); and *Loa loa*, the African eyeworm. Previously, we have demonstrated that patients infected with various filariae develop marked increases in serum levels of antigen-specific and polyclonal IgE¹⁹⁻²¹ and exhibit antigen-specific IgE-mediated activation of basophils with resultant release of histamine and IL-4.²¹

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Because treated patients may persistently harbor depots of filarial antigen in their soft tissues in the form of dead filarial worms, it may be that patients treated for filarial infections are unlikely to be completely free of filarial antigen. Nonetheless, by limiting our study population to patients who had not returned to filaria-endemic regions since initial treatment, we were able to study the duration of IgE responses in the clear absence of reinfection with pathogen. Our data provide evidence of the long-lived nature of helminth-specific IgE responses in the absence of environmental re-exposure to pathogen.

METHODS

Subjects

Filaria-infected patients and uninfected individuals were recruited from the Clinical Parasitology Unit (Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases [NIAID], National Institutes of Health, Bethesda, Md) and from the Department of Transfusion Medicine, National Institutes of Health, respectively, under protocols approved by the NIAID Institutional Review Board. Filarial infection was diagnosed by using (1) positive identification of appropriate parasite or parasite DNA in blood, skin snips, or tissue biopsy by microscopy or PCR, (2) positive antifilarial serology plus Calabar swellings and response to therapy for *L loa*, (3) positive circulating filarial antigen test for *W bancrofti*, (4) positive antifilarial serology plus either Mazzotti reaction or ophthalmologic examination consistent with onchocercal eye disease for *O volvulus*, or (5) meeting all of the established criteria for tropical pulmonary eosinophilia.²² Patients were classified as having acquired filarial infections as a consequence of being an immigrant to the United States, having visited friends and family abroad, or having been a traveler or temporary resident of a foreign country. In total, 8 uninfected individuals and 34 filaria-infected patients were included in the study: 27 with *L loa*, 4 with *O volvulus*, 2 with *W bancrofti*, and 1 coinfecting with *L loa* and *O volvulus* (see this article's Table E1 in the Online Repository at www.jacionline.org). All patients were treated with diethylcarbamazine for *L loa* and *W bancrofti* and with repeated courses of ivermectin for *O volvulus*. At follow-up, all patients were asymptomatic and had no laboratory evidence of active filarial infection, and none had traveled to filaria-endemic regions since their initial evaluation. Median time of follow-up evaluation was 33 months for all patients (range, 11-178 months).

Assessment of polyclonal and *Brugia malayi* antigen-specific IgE levels and IgE-producing cells

Quantification of polyclonal and *Brugia malayi* antigen (BmAg)-specific IgE serum levels was performed as previously described.²³ To determine frequencies of BmAg-specific and polyclonal IgE-producing cells, enzyme-linked immunospot assays (ELISPOTs) were performed by using PBMCs that had been cryopreserved and subsequently thawed. All pretreatment and posttreatment samples were run concurrently on the same plate. Immulon 4 plates (96-well; Thermo, Franklin, Mass) were coated overnight with either rat antihuman IgE (Biosource, Camarillo, Calif) at 5 µg/mL (for polyclonal IgE ELISPOT) or BmAg at 20 µg/mL (for BmAg-specific IgE ELISPOT). Plates were then washed and blocked overnight with PBS containing 5% BSA and 0.05% Tween. Serial dilutions of PBMC (2000 to 32,000 cells per well) in culture medium (Iscove's Dulbecco modified medium [Biofluids, Rockville, Md] supplemented with 10% heat-inactivated FCS

[HyClone, Logan, Utah], 1% L-glutamine [Biofluids], 1% insulin-transferrin-selenium medium [Biofluids], and 80 µg/mL gentamicin [BioWhittaker, Walkersville, Md]) were then placed in the wells in triplicate and incubated for 16 hours at 37°C. Plates were washed and incubated with biotinylated goat antihuman IgE, washed again, and then incubated with streptavidin alkaline phosphatase for 1 hour. 5-Bromo-4-chloro-3-indolyl phosphate/Nitro blue tetrazolium (BCIP/NBT) liquid substrate (Sigma Chemical, St Louis, Mo) was warmed to 37°C and then added to just-boiled 2.5% agarose at a 1.5:1 ratio by volume. Fifty microliters of this mixture was then rapidly added to each well by using a multichannel pipette with large-bore pipette tips. The plates were then placed in the dark, allowed to develop overnight, and read the next day with the use of a stereoscopic microscope. Each visible spot represented a B cell releasing either polyclonal or BmAg-specific IgE. Samples were not considered positive unless a clear halving of the average number of spots per well was observed after serial dilution; thus, the lowest limit of detection for the assay was 1 cell per 16,000 PBMCs (0.6 cells per 10,000 PBMCs).

Histamine release

Aliquots (200 µL) of heparinized whole blood diluted 1:7 with histamine release buffer (Beckman Coulter, Marseille Cedex, France) were incubated for 30 minutes at 37°C with buffer alone and with serial 10-fold dilutions of BmAg (from 100 to 1×10^{-7} µg/mL). One aliquot of blood from each patient was frozen and thawed twice to give a measure of total histamine stores. Tubes were then centrifuged for 5 minutes at 900g at 4°C and supernatants collected. Histamine concentrations were measured by using a histamine enzyme immunoassay kit (Beckman Coulter). Values obtained from different conditions were divided by total histamine to obtain percentages of total histamine released. Of note, all histamine release reactions were conducted on whole blood within 2 hours of blood drawing from the patient.

Cell preparation and fixation for intracellular flow cytometry

Heparinized venous blood was collected, and fresh PBMCs were separated by using a Ficoll diatrizoate gradient (lymphocyte separation medium; MP Biomedicals, Irvine, Calif). Cells were washed and then cultured at 2×10^6 per mL in culture medium with BmAg at 10 µg/mL, BmAg at 0.1 µg/mL, ionomycin (CalBiochem, La Jolla, Calif) at 1 µg/mL, or Staphylococcus enterotoxin B (Toxin Technologies, Sarasota, Fla) at 10 µg/mL, or in media alone. Brefeldin A (Sigma Chemical) was added at 20 µg/mL after 2 hours. After 6 hours, DNase (Sigma Chemical) was added at 666 U/mL and allowed to incubate for 5 minutes at 37°C. Cells were then transferred to 15-mL conical tubes, washed twice in PBS (BioWhittaker), and then fixed in PBS containing 4% paraformaldehyde (Sigma Chemical) for 5 minutes. Cells were then washed in PBS containing 0.1% BSA, resuspended in PBS/10% dimethyl sulfoxide (Fischer Scientific, Fair Lawn, NJ), and cryopreserved at -70°C.

Flow cytometry

Fixed cells were thawed, washed with PBS/0.1% BSA, and then permeabilized and blocked for 1 hour by incubating in PBS containing 0.1% saponin (CalBiochem)/1% BSA at 4°C. Cells were then stained with allophycocyanin-conjugated rat IgG₁ antihuman IL-4 (BD Pharmingen, San Jose, Calif) and either Cy-chrome-conjugated mouse IgG₁ antihuman CD4 for CD4⁺ T-cell analysis or, for basophil analysis, fluorescein isothiocyanate-conjugated goat antihuman IgE (Biosource), Cy-chrome-conjugated mouse IgG₁ antihuman CD2 (BD Pharmingen), tricolor-conjugated

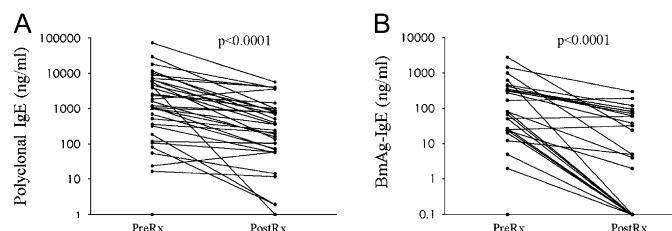


FIG 1. Polyclonal (A) and BmAg-specific (B) IgE levels before and after treatment of filarial infections.

mouse IgG_{2a} antihuman CD14 (Caltag, Burlingame, Calif), tricolor-conjugated mouse IgG₁ antihuman CD16 (Caltag), and Cy-chrome-conjugated mouse IgG_{1κ} antihuman CD19 (BD Pharmingen). Allophycocyanin-conjugated rat IgG₁ (BD Pharmingen) was used as an isotype control for IL-4 staining. After staining, cells were washed twice with PBS/0.1% saponin, resuspended in PBS, and analyzed by using a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, Calif) and CellQuest software (Becton Dickinson). Before patient samples were run, all antibodies were individually titrated with positive and negative control cells for optimal sensitivity and specificity.

Statistical analysis

The Wilcoxon signed-rank test was used to compare paired data, and the Mann-Whitney unpaired rank test for unpaired data. Correlations were derived by using the Spearman rank test. All statistics were performed by using StatView5 (SAS Institute, Cary, NC).

RESULTS

Patients have detectable circulating BmAg-specific IgE several years after treatment despite absence of re-exposure to filariae

Serum polyclonal and BmAg-specific IgE levels were obtained on all patients both before and after treatment (median interval between pretreatment and posttreatment values, 33 months; range, 11-178 months). As expected, most (26/34) filaria-infected patients had polyclonal IgE levels greater than the upper normal limit of 216 ng/mL (geometric mean [GM], 1113; range, 1-73,884 ng/mL), and 22 had levels greater than 1000 ng/mL. After treatment, the GM of the polyclonal IgE levels fell to within normal limits (GM, 190 ng/mL posttreatment; range, 1-5702; $P < .0001$ compared with pretreatment values), and only 7 patients had levels greater than 1000 ng/mL (Fig 1, A). Whereas none of the uninfected individuals tested positive for BmAg-specific IgE, 26 of 34 filaria-infected patients had detectable BmAg-specific IgE by ELISA. Although plasma levels of BmAg-specific IgE decreased significantly over time (pretreatment [preRx] GM, 21 ng/mL; posttreatment [postRx] GM, 1.9; $P < .0001$; Fig 1, B), the majority (17/26) of patients who had detectable BmAg-specific IgE pretreatment continued to have detectable BmAg-specific IgE after treatment. The likelihood of maintaining detectable BmAg-specific IgE was clearly dependent on pretreatment BmAg-specific IgE levels. None of the 8 patients with initial undetectable BmAg-specific IgE developed detectable BmAg-specific IgE at follow-

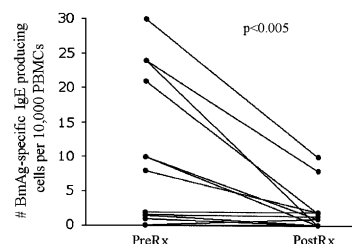


FIG 2. Frequencies of BmAg-specific IgE-releasing B cells before and after treatment of filarial infections.

up. Of the 26 patients with detectable BmAg-specific IgE before treatment, 13 had levels > 100 ng/mL, and 13 had levels < 100 ng/mL. All but 1 of the 13 with high pretreatment levels had detectable BmAg-specific IgE at follow-up, whereas only 5 of the 13 with low pretreatment levels remained detectable at follow-up. Remarkably, 6 of the 11 patients for whom posttreatment plasma samples were obtained at least 5 years after initial treatment had detectable BmAg-specific IgE at follow-up.

Patients have detectable circulating BmAg-specific IgE-producing cells several years after treatment

Frequencies of B cells producing either polyclonal or BmAg-specific IgE were measured by ELISPOT on patients 1, 2, 4 to 6, 10 to 17, and 19 (median interval between pretreatment and posttreatment testing, 43 months; range, 11-116 months). The GM polyclonal IgE-releasing cells in these patients decreased from 88 per 10,000 PBMCs before treatment to 63 per 10,000 PBMCs after treatment, although this difference was not statistically significant ($P > .5$; data not shown). In contrast, the frequency of BmAg-specific IgE-producing cells decreased more markedly after treatment, with the difference being statistically significant (GM preRx, 3.2 cells per 10,000 PBMCs; range, 0-30; GM postRx, 0.6 cells per 10,000 PBMCs; range, 0-10; $P < .005$; Fig 2). Notably, the majority of patients had detectable BmAg-specific IgE-producing cells both before and after treatment (12/14 with detectable BmAg-specific IgE-producing cells preRx and 8/14 postRx). Frequencies of cells producing polyclonal and BmAg-specific IgE as measured by ELISPOT correlated with plasma concentrations of polyclonal and BmAg-specific IgE as measured by ELISA (for polyclonal IgE, $\rho = 0.65$, $P < .001$; for BmAg-specific

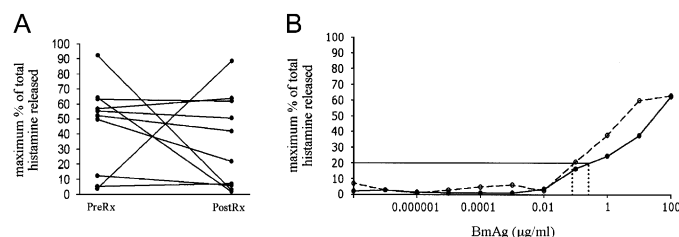


FIG 3. Histamine release from whole blood from filaria-infected patients after incubation with BmAg. **A**, Maximum percentage of histamine released by filaria-infected patients in response to BmAg before and several years after treatment. **B**, GM of percentage of total histamine released in whole blood in patients with positive histamine release reactions before (dashed line) and after (solid line) treatment. Dotted vertical lines denote the GM threshold BmAg concentrations necessary to induce >20% total histamine release.

IgE, $p = 0.42$, $P = .03$). Of note, none of the uninfected individuals had cells producing BmAg-specific IgE.

Basophils from filaria-infected patients release histamine several years after treatment

Filarial infections cause histamine release from basophils by antigen-specific IgE activation. To assess the ability of basophils to respond to parasite antigen years after treatment, histamine release reactions were done on patients 1 to 10 (median interval between pretreatment and posttreatment testing, 24 months; range, 11-37 months). The frequencies of patients exhibiting antigen-specific histamine release, defined as release of >20% of total histamine stores, did not change after treatment, because 7 patients released histamine in response to BmAg before treatment and 6 did so after treatment (Fig 3, A). Although the GM of maximum percentage of total histamine released did decrease several years after treatment (from GM 30% to GM 18%), this difference was not statistically significant ($P = .24$). In patients with positive histamine release, the threshold concentration of BmAg necessary to induce histamine release >20% was not significantly different after treatment compared with before treatment (0.20 µg/mL after treatment vs 0.09 µg/mL before treatment; $P = .52$; Fig 3, B). Of interest, total histamine stores decreased after active infection was treated (GM preRx, 824 nmol/L; range, 420-3980 vs GM postRx, 496 nmol/L; range, 38-2752), although this difference did not reach statistical significance ($P = .059$; data not shown).

Basophils from filaria-infected patients release IL-4 several years after treatment

In addition to release of histamine, antigen-specific IgE-mediated activation of basophils causes rapid release of IL-4. Previously, we have demonstrated that basophils from individuals with active filarial infections release IL-4 in response to BmAg.²¹ To determine whether basophils continue to release IL-4 in response to BmAg after the filarial infection has been treated, fresh PBMCs from patients 1 to 9 (median interval between pretreatment and posttreatment testing, 28 months; range, 11-37 months) were incubated with media, BmAg at 0.1 or 10 µg/mL, or ionomycin for 2 hours, fixed, and analyzed for cytokine

production by intracellular flow cytometry. Basophil populations were identified and gated on the basis of surface IgE positivity and negativity for CD2, CD14, CD16, and CD19, and the threshold for IL-4 positivity was established by using an isotype control (Fig 4, A).

Basophils from filaria-infected patients continued to release IL-4 in response to BmAg after treatment. After incubation with media alone, more basophils were IL-4 positive before treatment than after treatment (GM 0.7% preRx; range, 0.1% to 2.4%; GM 0.4% postRx; range, 0.1% to 1.9%), although this difference was not statistically significant ($P = .06$; data not shown). Stimulation with BmAg at both low (0.1 µg/mL) and high (10 µg/mL) concentrations resulted in higher frequencies of basophils releasing IL-4 both before and after treatment. At low concentrations of BmAg, more basophils released IL-4 before treatment (GM, 3.3%; range, 0.2% to 21.1%) than after treatment (GM, 1.1%; range, 0.1% to 10%), although this difference was not statistically significant ($P = .07$; Fig 4, B). After stimulation with a high concentration of BmAg, the frequencies of basophils releasing IL-4 were again higher before treatment than after treatment, but the difference was not as marked as that observed after stimulation with low concentrations of BmAg (GM, 2.5%; range, 0.3% to 9.1% preRx, compared with GM, 1.3%; range, 0.1% to 20% postRx; Fig 4, C). Again, the difference between frequencies of IL-4⁺ basophils before and after treatment was not statistically significant ($P = .76$).

The absolute numbers of basophils and CD4⁺ cells per 100,000 PBMCs releasing IL-4 in response to high concentrations of BmAg were equivalent after treatment (GM, 7.8 IL-4⁺ basophils per 100,000 PBMCs postRx vs GM, 6.0 IL-4⁺CD4⁺ cells per 100,000 PBMCs postRx; $P = .91$; data not shown).

DISCUSSION

This study demonstrates that filaria-specific IgE responses persist for years after treatment in the absence of re-exposure to filarial worms. Although levels decrease over time, filaria-specific IgE remains detectable in the plasma of most patients previously treated for filarial infection. Patients with high titers of antibody before

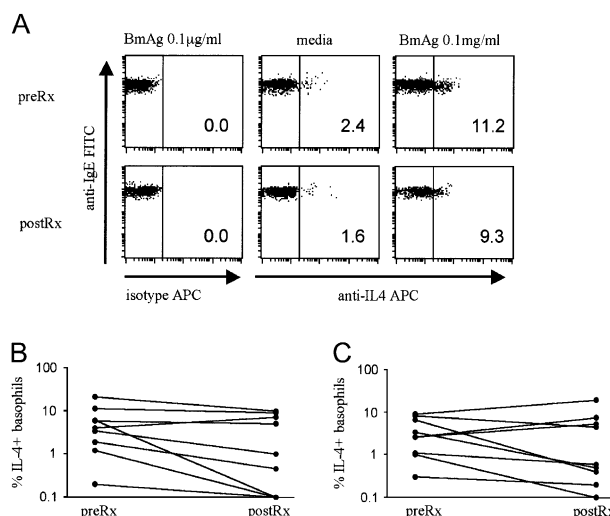


FIG 4. IL-4 release from basophils before and after treatment. **A**, Representative dot plots of basophil IL-4 release after 2 hours incubation with media or BmAg at 0.1 µg/mL. **B**, Percentages of IL-4⁺ basophils pretreatment and posttreatment after incubation with BmAg at 0.1 µg/mL. **C**, Percentages of IL-4⁺ basophils pretreatment and posttreatment after incubation with BmAg at 10 µg/mL. APC, Antigen-presenting cell; FITC, fluorescein isothiocyanate.

treatment were more likely to maintain detectable antigen-specific IgE than those with initially low titers. The presence of filaria-specific IgE can be quite long-lived, because more than half of the patients for whom posttreatment plasma samples were obtained at least 5 years after initial treatment had detectable filaria-specific IgE at follow-up. Similarly, although circulating numbers of filaria-specific IgE-producing cells decrease over time, they too were found to persist in the majority of patients after intervals of several years. The frequencies of circulating cells producing polyclonal and filaria-specific IgE correlated with plasma levels of these antibodies, consistent with previous studies showing correlation between numbers of IgE-producing cells and serum levels of IgE.^{24,25}

Notably, we found that the persistent filaria-specific IgE remains functionally relevant. Although there are nonspecific mechanisms of basophil activation, we have previously shown that basophil activation by BmAg occurs through cross-linking of specific IgE by parasite antigen, because individuals lacking BmAg-specific IgE do not release histamine in response to BmAg²¹ and because BmAg-specific histamine release from basophils of filaria-infected patients is abrogated after dissociation of IgE antibody from their high-affinity receptors on basophils (Unpublished data, June 2004). In the current study, we found that basophils from a majority of filaria-infected patients continue to release both histamine and IL-4 after stimulation with filarial antigen years after treatment of their infection. The magnitude of the basophil response to antigen may decrease over time, because both the maximum percentage of total histamine released and the frequencies of basophils releasing IL-4 in response to filarial antigen decreased after treatment, although neither difference was statistically significant. Of note, there was

no change in the sensitivity of basophils to filarial antigen, because the threshold concentration required to induce >20% of total histamine release was similar before and after treatment.

The conclusion that helminth-specific IgE responses persist for years after treatment of filarial infections in the absence of re-exposure to infection has several implications. Although deleterious in allergy and asthma, antigen-specific IgE appears to be protective in helminth infections. Epidemiologic studies reveal that helminth-specific IgE titers correlate with resistance to filariae,¹⁰ *Ascaris lumbricoides*,¹¹ and hookworm infections¹² in human beings. Protection against the larval stages of *O. volvulus* induced by immunization with *O. volvulus* third-stage larvae in mice is abolished after elimination of IgE by *in vivo* treatment with antibody against the Fcε portion of the murine H chain,⁷ and mice genetically deficient of IgE have increased susceptibility to infection with *Trichinella spiralis*⁸ and *Schistosoma mansoni*.⁹ Vaccine studies confirm the importance of antigen-specific IgE in the control of helminth infections, because vaccine protection in lambs vaccinated with *Haemonchus contortus* antigens²⁶ and in dogs vaccinated with recombinant *Ancylostoma* fusion proteins²⁷ correlates with high levels of helminth-specific IgE. On the basis of these vaccine studies, research is currently underway to study the feasibility of developing a hookworm vaccine that induces high-titer helminth-specific IgE.²⁸ The finding that filaria-specific IgE responses are long-lasting in the absence of re-exposure to infection suggests that such a vaccine, if effective, could potentially induce longstanding protection.

Our data also give credence to the hypothesis that basophils may serve to rapidly re-establish type 2 human immune responses after re-exposure to helminth pathogens. Of all PBMCs, only basophils have the ability to

release IL-4 early (almost immediately) in response to a stimulus,²⁹ releasing preformed IL-4 within 5 to 10 minutes of IgE surface crosslinking.³⁰ Furthermore, the quantity of IL-4 that basophils release is large, with 3 studies now showing that basophils release more IL-4 on a per cell basis than other cell types in response to antigenic challenge.^{21,31,32} In this study, we demonstrate not only that antigen-specific IgE persists after treatment of filarial infection but also that basophils continue to release IL-4 for several years after stimulation with filarial antigen. Because IL-4 causes naive T cells to differentiate toward a type 2 phenotype, this memory IL-4 release in response to helminth antigen may initiate a rapid re-establishment of the type 2 response to helminths on reinfection. In addition to skewing the T-cell response toward a type 2 phenotype, antigen-specific IgE-mediated activation of basophils may directly serve to induce greater IgE production, because basophils that express CD40 ligand and release IL-4 have the ability to induce B cells to switch to the IgE isotype.^{33,34} This concept of basophils as the primary initiators of a type 2 memory response is also supported by a recent study of type 2 immunity in 4get mice in which cells with an accessible IL-4 gene express a green fluorescent protein.³⁵

In terms of the effects of IgE responses in asthma and allergy, our findings suggest that even absolute allergen avoidance for many years would likely not result in loss of allergy. This conclusion is consistent with clinical experience, because patients can develop recurrent allergic symptoms despite allergen avoidance for many years.^{13,15,16} Indeed, a recently published clinical study reported that almost 1 in 5 people with a history of allergy to insect stings as a child has a systemic allergic reaction to an insect sting as long as 32 years later.³⁶ Unlike many other studies of longstanding IgE responses in human beings, ours is not confounded by the possibility of recurrent exposure to antigen, because filariae are not endemic in the United States and because we selected only patients who had not returned to countries endemic for filarial infection.

Our findings may also have implications for approaches to the treatment of allergic diseases. For example, several researchers are now developing strategies to treat allergy by inhibiting class switching³⁷⁻⁴⁰; however, as pointed out by Geha et al,⁴¹ such strategies will likely not block ongoing IgE production because of persistence of IgE-producing cells. Thus, the results of our study suggest that treatments that block solely IgE class switching may not adequately protect against allergy without being used in combination with blockade of current IgE-producing cells.

In interpreting our results, it is important to note that although the patients in our study were not re-exposed to filarial infection, it is likely that they may continue to harbor depots of filarial antigen in their soft tissues in the form of dead filarial worms. As such, our results can likely not be generalized to situations in which a person is truly free of antigen. Of course, whether humoral memory can even be maintained in the absence of antigen remains unknown. Antigen can clearly be stored in lymphoid

tissues for months to years in the form of immune complexes on follicular dendritic cells,^{42,43} and some researchers argue that persistent antigen is necessary to maintain long-lasting antibody responses.⁴⁴ Of interest, follicular dendritic cells, which can bind IgE with FcεRII (the low-affinity receptor for IgE), have the ability to trap and maintain IgE-antigen immune complexes in lymph nodes as well as IgG antigen-immune complexes.⁴⁵ Removal of follicular dendritic cells decreases recall antigen-specific IgE responses in *in vitro* mouse lymph node cultures,⁴⁶ suggesting that persistent antigen may be necessary for prolonged antigen-specific IgE production.

In this study, we did not determine the actual phenotype of the B cells producing antigen-specific IgE. Currently, persistent humoral immunity is believed to be caused by either polyclonal activation of memory B cells that are continually differentiating into plasma cells⁴⁷ or long-lived plasma cells that can survive in the bone marrow for >90 days without DNA synthesis⁴⁸ and continue to secrete antibody for >1 year in the absence of memory B cells.⁴⁹ Determining which of these mechanisms is responsible for long-term IgE memory could provide valuable insights into the immune response to helminths and into the development and maintenance of allergic diseases.

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REFERENCES

- Paul JR. Antibodies to three different antigenic types of poliomyelitis virus in sera from north Alaskan Eskimos. *Am J Hyg* 1951;54:275-85.
- Sawyer WA. The persistence of yellow fever immunity. *J Prev Med* 1931;5:413-28.
- Panum PL. Beobachtungen über das Maserncontagium. *Virchows Arch* 1847;1:492-515.
- Rossi CL, Takahashi EE, Partel CD, Teodoro LG, da Silva LJ. Total serum IgE and parasite-specific IgG and IgA antibodies in human strongyloidiasis. *Rev Inst Med Trop Sao Paulo* 1993;35:361-5.
- Estambale BB, Simonsen PE, Vennervald BJ, Knight R, Bwayo JJ. Bancroftian filariasis in Kwale District of Kenya, III: quantification of the IgE response in selected individuals from an endemic community. *Ann Trop Med Parasitol* 1995;89:287-95.
- Ramirez RM, Ceballos E, Alarcon de Noya B, Noya O, Bianco N. The immunopathology of human schistosomiasis, III: immunoglobulin isotype profiles and response to praziquantel. *Mem Inst Oswaldo Cruz* 1996;91:593-9.
- Abraham D, Leon O, Schnyder-Candrian S, Wang CC, Galatioto AM, Kerepesi LA, et al. Immunoglobulin E and eosinophil-dependent protective immunity to larval *Onchocerca volvulus* in mice immunized with irradiated larvae. *Infect Immun* 2004;72:810-7.
- Gurish MF, Bryce PJ, Tao H, Kisselgof AB, Thornton EM, Miller HR, et al. IgE enhances parasite clearance and regulates mast cell responses in mice infected with *Trichinella spiralis*. *J Immunol* 2004;172:1139-45.
- King CL, Xianli J, Malhotra I, Liu S, Mahmoud AA, Oettgen HC. Mice with a targeted deletion of the IgE gene have increased worm burdens and reduced granulomatous inflammation following primary infection with *Schistosoma mansoni*. *J Immunol* 1997;158:294-300.
- Lobos E, Nutman TB, Hothersall JS, Moncada S. Elevated immunoglobulin E against recombinant *Brugia malayi* γ-glutamyl transpeptidase in patients with bancroftian filariasis: association with tropical pulmonary eosinophilia or putative immunity. *Infect Immun* 2003;71:747-53.
- Turner JD, Faulkner H, Kamgno J, Kennedy MW, Behnke J, Boussinesq M, et al. Allergen-specific IgE and IgG4 are markers of resistance and

- susceptibility in a human intestinal nematode infection. *Microbes Infect* 2005;7:990-6.
12. Bethony J, Loukas A, Smout M, Brooker S, Mendez S, Plieskatt J, et al. Antibodies against a secreted protein from hookworm larvae reduce the intensity of hookworm infection in humans and vaccinated laboratory animals. *FASEB J* 2005;19:1743-5.
13. Blanca M, Torres MJ, Garcia JJ, Romano A, Mayorga C, de Ramon E, et al. Natural evolution of skin test sensitivity in patients allergic to β -lactam antibiotics. *J Allergy Clin Immunol* 1999;103:918-24.
14. Fisher MM, Baldo BA. Persistence of allergy to anaesthetic drugs. *Anaesth Intensive Care* 1992;20:143-6.
15. Bernardini R, Novembre E, Lombardi E, Pucci N, Rossi ME, Vierucci A. Long persistence of IgE antibody to cefaclor. *Allergy* 2000;55:984-5.
16. Lemiere C. Persistence of bronchial reactivity to occupational agents after removal from exposure and identification of associated factors. *Ann Allergy Asthma Immunol* 2003;90:52-5.
17. Chapa-Ruiz MR, Gonzalez-Pantaleon D, Morales-Galan A, Contreras-Ramos A, Salinas-Tobon MR, Martinez YZR. A follow-up study of the human class and subclass antibody response developed against the adult stage of *Trichinella spiralis*. *Parasite* 2001;8:S163-7.
18. Steel C, Lujan-Trangay A, Gonzalez-Peralta C, Zea-Flores G, Nutman TB. Immunologic responses to repeated ivermectin treatment in patients with onchocerciasis. *J Infect Dis* 1991;164:581-7.
19. Lee SJ, Francis HL, Awadzi K, Ottesen EA, Nutman TB. Changes in antibody profile after treatment of human onchocerciasis. *J Infect Dis* 1990;162:529-33.
20. McCarthy JS, Ottesen EA, Nutman TB. Onchocerciasis in endemic and nonendemic populations: differences in clinical presentation and immunologic findings. *J Infect Dis* 1994;170:736-41.
21. Mitre E, Taylor RT, Kubofcik J, Nutman TB. Parasite antigen-driven basophils are a major source of IL-4 in human filarial infections. *J Immunol* 2004;172:2439-45.
22. Ottesen EA, Nutman TB. Tropical pulmonary eosinophilia. *Annu Rev Med* 1992;43:417-24.
23. Mitre E, Norwood S, Nutman TB. Saturation of immunoglobulin E (IgE) binding sites by polyclonal IgE does not explain the protective effect of helminth infections against atopy. *Infect Immun* 2005;73:4106-11.
24. Horst A, Hunzelmann N, Arce S, Herber M, Manz RA, Radbruch A, et al. Detection and characterization of plasma cells in peripheral blood: correlation of IgE⁺ plasma cell frequency with IgE serum titre. *Clin Exp Immunol* 2002;130:370-8.
25. King CL, Poindexter RW, Raganathan J, Fleisher TA, Ottesen EA, Nutman TB. Frequency analysis of IgE-secreting B lymphocytes in persons with normal or elevated serum IgE levels. *J Immunol* 1991;146:1478-83.
26. Kooyman FN, Schallig HD, Van Leeuwen MA, MacKellar A, Huntley JF, Cornelissen AW, et al. Protection in lambs vaccinated with *Haemonchus contortus* antigens is age related, and correlates with IgE rather than IgG1 antibody. *Parasite Immunol* 2000;22:13-20.
27. Hotez PJ, Ashcom J, Bin Z, Bethony J, Williamson A, Hawdon JM, et al. Effect of vaccinations with recombinant fusion proteins on *Ancylostoma caninum* habitat selection in the canine intestine. *J Parasitol* 2002;88:684-90.
28. Hotez PJ, Zhan B, Bethony JM, Loukas A, Williamson A, Goud GN, et al. Progress in the development of a recombinant vaccine for human hookworm disease: the Human Hookworm Vaccine Initiative. *Int J Parasitol* 2003;33:1245-58.
29. Gibbs BF, Haas H, Wolff HH, Grabbe J. Early IgE-dependent release of IL-4 and IL-13 from leukocytes is restricted to basophils: a comparison with other granulocytes and mononuclear cells. *Inflamm Res* 2000;49(suppl 1):S9-10.
30. Gibbs BF, Haas H, Falcone FH, Albrecht C, Vollrath IB, Noll T, et al. Purified human peripheral blood basophils release interleukin-13 and preformed interleukin-4 following immunological activation. *Eur J Immunol* 1996;26:2493-8.
31. Poorafshar M, Helmby H, Troye-Blomberg M, Hellman L. MMCP-8, the first lineage-specific differentiation marker for mouse basophils. Elevated numbers of potent IL-4-producing and MMCP-8-positive cells in spleens of malaria-infected mice. *Eur J Immunol* 2000;30:2660-8.
32. Aoki I, Kinzer C, Shirai A, Paul WE, Klinman DM. IgE receptor-positive non-B/non-T cells dominate the production of interleukin 4 and interleukin 6 in immunized mice. *Proc Natl Acad Sci U S A* 1995;92:2534-8.
33. Gauchat JF, Henchoz S, Mazzei G, Aubry JP, Brunner T, Blasey H, et al. Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature* 1993;365:340-3.
34. Yanagihara Y, Kajiwaru K, Basaki Y, Ikizawa K, Ebisawa M, Ra C, et al. Cultured basophils but not cultured mast cells induce human IgE synthesis in B cells after immunologic stimulation. *Clin Exp Immunol* 1998;111:136-43.
35. Khodoun MV, Orekhova T, Potter C, Morris S, Finkelman FD. Basophils initiate IL-4 production during a memory T-dependent response. *J Exp Med* 2004;200:857-70.
36. Golden DB, Kagey-Sobotka A, Norman PS, Hamilton RG, Lichtenstein LM. Outcomes of allergy to insect stings in children, with and without venom immunotherapy. *N Engl J Med* 2004;351:668-74.
37. Burger MS, Zuleger CL, Chu Q, Gao X, Payne LG, Chen D. An antigen-independent but not antigen-specific T_H1 response provides protection in the murine airway inflammation model. *J Allergy Clin Immunol* 2004;114:1301-8.
38. Trujillo-Vargas CM, Mayer KD, Bickert T, Palmetshofer A, Grunewald S, Ramirez-Pineda JR, et al. Vaccinations with T-helper type 1 directing adjuvants have different suppressive effects on the development of allergen-induced T-helper type 2 responses. *Clin Exp Allergy* 2005;35:1003-13.
39. Horner AA, Raz E. Immunostimulatory sequence oligodeoxynucleotide-based vaccination and immunomodulation: two unique but complementary strategies for the treatment of allergic diseases. *J Allergy Clin Immunol* 2002;110:706-12.
40. Sur S, Wild JS, Choudhury BK, Sur N, Alam R, Klinman DM. Long term prevention of allergic lung inflammation in a mouse model of asthma by CpG oligodeoxynucleotides. *J Immunol* 1999;162:6284-93.
41. Geha RS, Jabara HH, Brodeur SR. The regulation of immunoglobulin E class-switch recombination. *Nat Rev Immunol* 2003;3:721-32.
42. Mandel TE, Phipps RP, Abbot AP, Tew JG. Long-term antigen retention by dendritic cells in the popliteal lymph node of immunized mice. *Immunology* 1981;43:353-62.
43. Tew JG, Wu J, Qin D, Helm S, Burton GF, Szakal AK. Follicular dendritic cells and presentation of antigen and costimulatory signals to B cells. *Immunol Rev* 1997;156:39-52.
44. Gray D. A role for antigen in the maintenance of immunological memory. *Nat Rev Immunol* 2002;2:60-5.
45. Maeda K, Burton GF, Padgett DA, Conrad DH, Huff TF, Masuda A, et al. Murine follicular dendritic cells and low affinity Fc receptors for IgE (Fc ϵ R2). *J Immunol* 1992;148:2340-7.
46. Helm SL, Burton GF, Szakal AK, Tew JG. Follicular dendritic cells and the maintenance of IgE responses. *Eur J Immunol* 1995;25:2362-9.
47. Bernasconi NL, Traggiai E, Lanzavecchia A. Maintenance of serological memory by polyclonal activation of human memory B cells. *Science* 2002;298:2199-202.
48. Manz RA, Thiel A, Radbruch A. Lifetime of plasma cells in the bone marrow. *Nature* 1997;388:133-4.
49. Slifka MK, Antia R, Whitmire JK, Ahmed R. Humoral immunity due to long-lived plasma cells. *Immunity* 1998;8:363-72.