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Human IgE mAbs Identify Major Antigens of Parasitic Worm Infection

Azadeh Hadadianpour[1,2], **Jacob Daniel**[1,2], **Jian Zhang**[1,2], **Benjamin W. Spiller**[2,3], **Asta Makaraviciute**[4], **Åsa M. DeWitt**[4], **Heather S. Walden**[5], **Robert G. Hamilton**[6], **R. Stokes Peebles Jr.**[1,2], **Thomas B. Nutman**[7], **Scott A. Smith**[1,2,*]

[1]Department of Medicine, Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN, 37232

[2]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN, 37232

[3]Department of Pharmacology, Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN, 37232

[4]Special Allergen Service, Thermo Fisher Scientific, Uppsala, Sweden

[5]Department of Comparative, Diagnostic and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, FL, 32610

[6]Dermatology, Allergy and Clinical Immunology Laboratory, Johns Hopkins University School of Medicine, Baltimore, MD, 21224

[7]Laboratory of Parasitic Diseases, NIAID, National Institutes of Health; Bethesda, Maryland, United States of America

Abstract

Rationale: Much of our understanding of the targets of IgE comes from studies of allergy, though little is known about the natural immunogenic targets seen following parasitic worm infections. The use of human monoclonal antibodies (mAbs) allow for an unbiased, comprehensive

[*]**Corresponding Author:** Division of Infectious Diseases, Vanderbilt University Medical Center, A2210 Medical Center North, 1161 21st Avenue South, Nashville, TN 37232-2582, scott.smith.1@vumc.org, Phone: 615-343-4028.

AUTHOR CONTRIBUTIONS

SAS, AH, and JD isolated hybridomas, purified antibodies, and sequenced mAb clones. AH and JD performed antigen discovery and binding experiments. AM and ÅMD developed *B. malayi* ImmunoCAP and performed antibody binding analysis. TBN recruited patients and assembled the cohort. RSP and JZ devised and performed mouse studies. AH and SAS prepared the manuscript. All authors revised and approved the final version of the manuscript.

Author conflicts of interest:

Scott A. Smith is an inventor on a patent (US 10,908,168) describing a method to make IgE antibodies, including those related to this work.

RESOURCE AVAILABILITY

All IgE monoclonal antibodies generated in this study are available from the lead contact with a completed materials transfer agreement.

characterization of the immunodominant antigens targeted by IgE in conditions (e.g., allergy, helminth infection) associated with elevated levels of IgE.

Methods: Using human hybridoma technology to immortalize IgE encoding B-cells from peripheral blood of subjects with filarial infections and elevated IgE, we generated naturally-occurring human IgE mAbs. B-cell cultures were screened in an unbiased manner for IgE production without regard to specificity. Isolated IgE mAbs then were tested for binding to *Brugia malayi* somatic extracts (BmA) using ImmunoCAP, immunoblot, and ELISA. Immunoprecipitation followed by mass spectrometry proteomics was used to identify helminth antigens that were then expressed in *E. coli* for IgE binding characterization.

Results: We isolated 56 discrete IgE mAbs from 7 individuals with filarial infections. From these mAbs, we were able to definitively identify 19 filarial antigens. All IgE mAbs targeted filarial excreted/secretory (E/S) proteins, including a family of previously uncharacterized proteins. Interestingly, the transthyretin-related antigens acted as the dominant inducer of the filaria-specific IgE antibody response. These filaria-specific IgE mAbs were potent inducers of anaphylaxis when passively administered to hFcεRI-expressing mice.

Conclusions: We have generated human hybridomas secreting naturally-occurring helminth-specific IgE mAbs from filarial-infected subjects. This work provides much needed insights into the ontogeny of the helminth-induced immune response/IgE antibody response.

CAPSULE SUMMARY

This is the first description of the dominant antigens targeted by the IgE antibody response during human parasitic worm infection.

Keywords

IgE; antibodies; monoclonal; helminth; parasite

INTRODUCTION

There is intense interest in the immunogenic triggers responsible for the rising epidemic of IgE-mediated allergic diseases; however, the ontogeny of the IgE antibody response in helminth infection has been poorly studied. While elevated serum levels of IgE antibodies are commonly found in tissue-invasive helminth infections, including the filarial nematodes that cause lymphatic filariasis, loiasis, and onchocerciasis[1], there is limited understanding of antigenic triggers underlying this response and are opposing data published regarding the protective function of IgE. Epidemiological data supporting the protective function of IgE show that anti-parasite IgE responses are associated with a degree of immune-mediated protection in humans infected with hookworms[2,3], Trichuris[4], Ascaris[5,6] and schistosomes[7-13]. However, much uncertainty exists as to the primary antigenic targets of IgE and its role in immune defense. Early serologic studies characterize IgE antibodies as ‘nonspecific’ (non-antigen specific), implying that IgE antibodies do not protect hosts in the course of parasitic infection [14]. Others have focused on allergen-like proteins such as tropomyosin to explore overlapping IgE responses between helminth infection and allergic diseases, these helminthic allergen-like proteins are included in the allergens database [15-17].

Still other studies report the presence of helminth-specific oligosaccharides and classical CCD (cross-reactive carbohydrate determinants) that are the targets for IgE antibodies in helminth infection, but there is relatively little evidence regarding the significance of IgE antibodies to these epitopes[18,19]. The overarching limitations of these studies stem from the difficulty of studying IgE using human sera, given the broad repertoire and exceedingly low concentration of antigen-specific IgE. An important new way to dissect the function of IgE in the context of human helminth infection is to create naturally occurring IgE monoclonal antibodies (mAbs). Our group has developed methods that allow for the generation of stable human hybridomas from the very rare population of helminth-specific IgE-encoding B cells in peripheral blood of infected human subjects. Here, we performed an unbiased study of IgE antibodies associated with filarial infection and identified several filarial antigens targeted by the IgE response, which have strong diagnostic and vaccine potential. Moreover, we suggest that IgE antibodies can act as an early detection system that can trigger a type one hypersensitivity response to constrain the parasite at a vulnerable early stage.

Filarial nematodes are threadlike worms with complex life cycles in which the adult worms reside in lymphatic or subcutaneous tissue of their host. These parasites are responsible for causing lymphatic filariasis (LF; *Wuchereria bancrofti, Brugia malayi, Brugi timori*), onchocericasis (*O. volvulus*) and/or loiasis (*Loa loa*). These chronic infections are responsible for an extraordinary degree of morbidity and collectively affect more than 180 million people worldwide. The canonical host immune response to these particular tissue-invasive helminths is of the T-helper 2 (Th2) type and involves the production of cytokines, interleukin (IL)-4, IL-5, IL-9, IL-10, and IL-13; the antibody isotypes immunoglobulin G1 (IgG1), IgG4, and IgE; and expanded populations of eosinophils, basophils, mast cells, type 2 innate lymphoid cells, and alternatively activated macrophages[20]. Interestingly, filarial infection in travelers and tropical pulmonary eosinophilia (TPE; an unusual syndrome seen in *W. bancrofti* and *B. malayi* infection) are associated with both extraordinarily elevated IgE levels and “allergic” symptomatology[21]. Thus, having insights into the antigens driving IgE and mediating pathology is of paramount interest.

METHODS

Data reporting

No statistical methods were used to predetermine sample size. Experiments were not randomized, and investigators were not blinded to allocation during experiments and outcome assessment.

Research subjects

We analyzed seven subjects with a clinical history of lymphatic filariasis, loiasis, or onchocerciasis, two diagnosed with TPE. Relevant clinical information is summarized in Supplementary Table 1. The study was approved by the Institutional Review Board of Vanderbilt University Medical Center (IRB 170308). Samples were obtained after written informed consent. Cells remained stored in liquid nitrogen until use.

Human hybridoma generation and variable gene sequencing

IgE-secreting human hybridomas were generated using methodology described previously[22]; please see supplemental methods for additional details. Purified monoclonal antibodies, expressed by large scale growth of human hybridomas in serum free culture medium, were then used for target identification, binding analysis, and anaphylaxis assessments. IgE antibody variable gene sequences were determined using RT-PCR amplification of total RNA extracted from 1 million clonal IgE-expressing human hybridoma cells, please see supplemental methods for additional information.

Parasite material

Adult parasites, infective larvae (L3), L4 larvae and microfilariae of *B. malayi* were obtained from the NIAID/NIH Filariasis Research Reagent Repository Center (FR3; Athens, GA; www.filariasiscenter.org). Additional adult *B. malayi* were purchased from TRS Labs (Athens, GA). *D. immitis* worms were also a source of filarial antigens: adult male and female *D. immitis* parasites were obtained from surgical removal in severe cases of heartworm infection in canines. Soluble antigen from adult *B. malayi* and *D. immitis* worms was prepared by grinding frozen worms to a fine powder in liquid nitrogen using a mortar and pestle (cryogenic grinding). The homogenized powder was suspended in phosphate buffer saline (PBS 0.05 M, pH 7.2) containing Halt protease inhibitor cocktail (Thermo Scientific, 78429) rocking for 1 h at 4°C. Proteins soluble in PBS were recovered by centrifugation (15,000 g) at 4°C for 30 min. Protein concentration was measured by absorbance at UV280. Filarial worm extracts were aliquoted and stored at −20°C until use. Extracts then were used in binding analysis as well as identification of target antigens using immunoprecipitation and mass spectrometry; please see supplemental methods.

ImmunoCAP, ELISA, and immunoblot analysis

Prototype ImmunoCAP *B. malayi* tests were developed for research use from a *B. malayi* somatic extract. Analytical characteristics of ImmunoCAP tests were determined and an accelerated stability study was performed. The test was used to screen reactivity of human IgE mAbs (at an approximate concentration of 1-10 μg/mL) against *B. malayi.* An antibody was considered positive if it bound to *B. malayi* in ImmunoCAP (>1.0 kU_A/L). We note that the assigned cutoff for ImmunoCAP positivity is much higher than the standard cutoff for serum analysis, as we used excess IgE antibody and antigen concentration is the limiting factor. For a detailed description of ELISA and immunoblotting procedures used in the identification and characterization of IgE mAbs, please see supplemental methods.

Expression and analysis of recombinant antigens

Recombinant his-tagged filarial antigens were expressed in SHuffle® T7 competent *E. coli* bacteria. For detailed description of the gene sequences used for expression, please see supplemental methods. Recombinant protein was purified using TALON metal affinity resin, followed by desalting to remove imidazole and to exchange buffer to PBS. Purified proteins were assessed for purity and appropriate molecular weight by SDS–PAGE.

Human FcεRI–transgenic mouse anaphylaxis

Mouse studies were carried out in accordance with recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Human FcεRI–transgenic mice [B6.Cg-Fcer1a^{tm1Knt} Tg(FCER1A)1Bhk/J] were purchased from The Jackson Laboratory (stock 010506), bred and genotyped. These mice carry 2 gene mutations: the human Fc fragment of IgE receptor α polypeptide (FCER1A) under control of the human FCER1A promoter and a mutation targeting Fcer1a^{tm1Knt}, blocking expression of murine FCER1A[23]. Human IgE can induce anaphylaxis in mice hemizygous for the transgene and homozygous for targeted deletion of mouse FcεRI. Transgenic mice were sensitized by i.p. injection of 100 μg total IgE and challenged by i.p. injection of 50 μg purified recombinant antigen. Changes in mouse core body temperature were monitored over 90 min using implanted temperature probes.

Statistics

For ELISA studies, mean relative fluorescence units were calculated using three independently performed assays performed in triplicate. The descriptive statistics mean ± SEM or mean ± SD were determined for continuous variables as noted. Statistical analysis was performed using Prism v9.0 (GraphPad) *software*. In mouse studies, the comparison of temperature-change curves was performed independently for each antigen challenge and at each time point using paired 2-tailed t test, assuming unequal variance (Microsoft Excel Office Professional Plus 2016). Time points with calculated P values less than 0.05 were labeled and considered significant. Error bars for mouse temperature measurements represent SEM.

RESULTS

IgE-Positive B Cells are Present in Blood of Subjects with Filariasis

To date, few studies have directly examined the B cells that produce IgE antibodies in humans[22,24]. Thus, we asked whether IgE-producing B cells are present in blood of subjects infected with filarial nematodes and, if so, could they be used to generate human IgE mAbs. To do so, we obtained frozen peripheral blood mononuclear cells (PBMC) from subjects with lymphatic filariasis, TPE, loiasis, or onchocerciasis. Using our previously published human hybridoma methodology[22] (Supplemental Data Fig. 1a), we generated 56 IgE mAbs from B cells from 7 individuals without selection for antigenic specificity (See Table 1). Those observations indicate a frequency of IgE-expressing B cells in circulation of these subjects ranging from 6-14 cells per 10 million PBMCs, slightly lower than the number of IgE-expressing B cells we previously reported for subjects with allergic bronchopulmonary aspergillosis[22].

Identification of Filaria-Specific IgE Antibodies

We next determined whether candidate IgE mAbs were directed against filarial antigens (Supplemental Data Fig. 1b). To do so we produced whole-worm somatic extracts from *B. malayi* or *Dirofilaria immitis*, which are responsible for filariasis in humans or dogs, respectively, and tested mAb reactivity against each extract using three complementary

assays—immunoblotting, ELISA and *B. malayi* ImmunoCAP. Of the 56 human mAb candidates, 26 were positive in at least one screen and some in all three (Fig. 1a and Supplementary Table 1). Of the 26 antibodies with detectable binding, 9 (35%) were immunoblot-positive against *B. malayi* with 5 (19%)also capable of blotting *D. immitis*. Representative blot images showing different banding patterns observed for candidate mAbs are shown for *B. malayi* in Fig. 1b and *D. immitis* in Fig. 1c. MAb 2E6, which had the greatest positivity in *B. malayi* ImmunoCAP, also showed robust positivity on immunoblots and exhibited a ladder-like pattern ranging from ~14 kDa to >250 kDa. Interestingly, except for mAb 2E6, all bands detected by immunoblot analysis were <30 kDa in size.

Of note, of the 26 filaria-specific IgE mAbs, 15 were obtained from the two subjects with TPE (15 of their 23 mAbs, 65%). Because *W. bancrofti* (which is responsible for 90% of LF infections), *Loa loa* and *Onchocerca volvulus* cannot be maintained in conventional mouse strains, we could not screen our IgE mAbs against these filarial somatic extracts and thus could not identify *Wuchereria*-specific, *Onchocerca*-specific, or *Loa*-specific IgE mAbs in our panel. Of the 26 filaria-specific IgE mAbs, only 13 showed cross-reactivity towards dog heartworm (Supplementary Table 1). Taken together this suggests that the IgE antibody response is highly specific to antigens of the infecting filarial worm, though, at the protein level there is a high (>90%) average sequence identity across the filarial species that are pathogenic for humans[25].

Given reports that IgE from patients with parasitic infection cross-react with common allergens[18,19], we asked whether our 26 candidate antibodies obtained from filaria-infected subjects cross-reacted with common allergen proteins. To do so we analyzed all 26 using an ImmunoCAP ISAC allergen microarray containing 112 purified allergens. We observed no significant reactivity of anti-filarial IgE to any allergen tested (Supplemental Data Fig. 2), suggesting that the IgE antibody response induced during filarial infection are highly filarial-specific.

The Antigenic Landscape of the Anti-filaria IgE Antibody Response

To assess antibody specificity, we focused on the 26 antibodies that showed reactivity to a filarial worm extract in multiple assays, as described above. To identify antigen targets, we independently immobilized the 26 candidate mAbs to magnetic beads and performed immunoprecipitation (IP) of *B. malayi* and *D. immitis* somatic extracts and subsequent mass spectrometry analysis. Immunoprecipitates with human IgE mAbs specific for irrelevant antigens from mold and peanut (mAbs 21E2, 16A8) served as specificity controls. IP was followed by immunoblotting to confirm antigen enrichment, as can be seen by examples shown in Fig. 2a. In some cases, such as with mAb 5H1 and 9C1, filarial antigen was at such a low concentration that IP resulted in complete depletion of antigen. In other cases, such as with mAb 2E6, a very high concentration of the filarial antigen resulted in saturation, as antigen can be seen in the post-IP material.

Overall, 16 IgE mAbs were able to immunoprecipitate a filarial antigen; we identified 14 unique antigens (Supplemental Table 1). MAbs 1A5, 5H1, 9C1 and 14B2 precipitated transthyretin-related protein (Fig. 2b), a 16 kDa nematode-secreted protein and member of the “(TTR)-like” family of proteins. Proteins of this family are reportedly prominent

components of the secretome of several parasites including *B. malayi*[26], *D. immitis*[27], *T. colubriformis*[28], and *C. elegans*[29]. Their function is largely unknown, but a TTR protein from the plant parasite *M. javanica* reportedly interferes with the host immune response and promotes parasitism[30]. MAb 12D4 precipitated WbSXP-1 (Fig. 2b), a 14 kDa nematode-specific secreted protein (also known as Wb14 antigen) previously reported as a target of IgG4 antibody[31] and an antigen used in multiple diagnostic tests[32,33]. WbSXP-1 homologues are present in *Anisakis*[34] and have been pursued as vaccine candidates against *Ascaris*[35]. MAb 11H12 precipitated a 24 kDa nematode-specific secreted protein with no known function (24 kDa SPro) (Fig. 2b). Although there were no NCBI sequences reported for the *W. bancrofti* homologue of this protein, a similar protein in *D. immitis* named 22–24 kDa excretory-secretory 22U protein has been reported[36]. MAb 4E9 precipitated Macrophage Migration Inhibitory Factor (MIF) (Fig. 2b), a worm homologue of human MIF, a proinflammatory cytokine[37]. Filarial MIF functions in immunoregulation and possibly pathogenesis via attracting host monocytes/macrophages to modify host immune responses and promote parasite survival. Finally, mAb 2E6, which showed the most intense signal in all screening assays, precipitated the polyprotein ladder-like protein or gp15/400 (Fig. 1a, b, 2a), a ~400 kDa nematode polyprotein allergen/antigen (NPA-1) with 20 tandemly arranged repeat subunits of 132 amino acid residues. *B. malayi* gp15/400 protein is associated with the worm surface and distributed in all tissues of the parasite[38]. The presence of gp15/400 protein in the *B. malayi* secretome was confirmed by immunoblot of culture media used to keep worms alive for 7 days (Supplemental Data Fig. 3). While antibody 2E6 is specific for *B. malayi* and *W. bancrofti* gp15/400, it did not show cross-reactivity with *D. immitis* gp15/400.

To confirm IgE mAb specificity, we expressed recombinant forms of 19 filarial antigens (SXP, 24 kDa SPro, MIF, gp15/400, and 15 different TTR proteins) in bacteria using the *W. bancrofti* sequence and tested binding by ELISA (Fig. 2c). If IgE mAbs showed positivity in an immunoblot of somatic extract, we also performed immunoblotting (Fig. 2d). When we were able to express and purify antigen protein in sufficient quantities, we calculated the EC50 for each antibody (Fig. 2c, Table 2). EC50 measurements ranged from single ng/mL, such as with mAb 11H12, to nearly μg/mL concentrations. Antibodies 5H1 and 18H7 showed a range of EC50 binding values to different TTR proteins, demonstrating their varied breadth of cross-reactivity within this protein family (Fig. 2c). Another interesting feature of TTR protein-specific mAbs is their varied tendency to bind TTR dimers (see Fig. 2d). MAb 9C1 binds preferentially to dimeric TTR, 18H7 prefers monomeric TTR, and 5H1 binds equally to both.

We next tested the 30 IgE mAbs from the original 56 that had not tested positive in reactivity tests against each recombinant antigen to ensure that no antibody was missed due to its specificity for *W. bancrofti*. MAb 4E1, for example, was found specific for WbSXP-1 but negative in all primary extract screens. Unlike mAb 12D4, 4E1 is highly specific to *W. bancrofti* and does not cross-react with *B. malayi* or *D. immitis* SXP-1 proteins (Supplemental Data Fig. 4). Finally, two mAbs, 5D2 and 7G12, were positive by ELISA and ImmunoCAP but negative in IP analysis (Supplemental Table 1). MAb 5D2 and 4G8 showed weak binding to several allergen proteins based on the ImmunoCAP ISAC assay (Supplemental Data Fig. 2), suggesting poly-reactivity to a carbohydrate epitope.

Overall, these results indicate that the humoral immune response to filarial infection targets a restricted set of filarial-specific E/S proteins.

TTR Proteins are Immunodominant Filarial Antigens with Diagnostic Potential

TTR proteins identified here as target antigens of multiple filaria-specific IgE mAbs have not been previously reported as filarial immunogens. Thus, we asked whether our panel of filaria-specific IgE included mAbs specific to other TTR proteins. To do so, we first assembled a phylogenetic tree using WbTTR protein sequences (total of 25 encoded by *W. bancrofti*) collected from NCBI (Supplemental Data Fig. 5). TTR family proteins shared a low degree of identity, ranging from 18.1% to 70.1% when compared in pairwise fashion (Supplemental Data Fig. 5b). Since this family of proteins has not been well studied, the functions of which remain unknown, we are unable to speculates as to the purpose for the large number and tremendous variability of the TTR proteins excreted by filarial helminths.

Next, we chose 15 TTR proteins as representatives of the family and expressed each as a his-tagged fusion protein for IgE mAb testing in ELISA and immunoblotting. Binding analysis of the 15 TTR proteins with 10 IgE mAbs revealed that some antibodies were highly specific to one TTR protein (Fig. 3a), while others showed varying degrees of cross-reactivity across different TTR protein family members (Fig. 3a, Supplemental Data Fig. 6). Of note, two IgE mAbs, 5H1 and 18H7, broadly cross-reacted with 13 different TTR proteins despite minimal amino acid conservation (Fig 2c, 3a Supplemental Data Fig. 5b, 6, 7). Antibodies targeting TTR protein antigens made up the greatest proportion of our panel of filaria-specific antibodies (Fig. 3b). Overall, these results indicate that TTR family proteins are the most dominant filarial antigens targeted by the human IgE antibody response (Fig. 3b, Table 2).

We next tested sera of subjects with filarial infection for the presence of filaria-specific IgE antibodies using immunoblotting of 17 recombinant filarial antigens. We chose 3 subjects (P1, P3, P5) from whom IgE mAbs were generated from their PBMCs in this study, and 2 subjects (P8, P9) in which no PBMCs were used and no IgE mAbs were developed, to assess each for the presence of specific antibodies. Immunoblotting not only verified the presence of antibodies with the same specificity as those of our IgE mAbs in the serum of subjects from whom mAbs were developed, but also confirmed presence of antibodies with similar specificities in other filarial worm infected subjects. Each subject sample showed a distinct pattern of reactivity predominantly to TTR family proteins (Fig. 3c, Supplemental Data Fig. 8), confirming that antigens identified here could be used to develop species-specific diagnostics.

Filaria-Specific IgE mAbs Exhibit a High Degree of Somatic Hypermutation (SHM)

To evaluate sequence diversity and degree of somatic hypermutation, we performed sequence analysis of 16 filaria-specific IgE hybridoma clones (Fig. 4). That analysis revealed 16 unique Vh–Jh–CDRH3–Vl–Jl–CDRL3 clonotypes (Supplementary Data Fig. 9). Filaria-specific IgE antibodies varied widely in their antibody variable region (VH and VL) germline gene usage, with no statistically significant over-representation of any germline gene (Fig. 4a). They also varied in the lengths of VH and VL complementarity-determining

region 3 (CDR3), but relative to other isotypes, IgE B cells show similar length distributions of CDR3 amino acids in the heavy and light chains[39] (Fig. 4b, c). Filaria-specific IgE mAbs exhibited a high degree of SHM in VH and VL genes (Fig. 4d), which contained an average of 23 and 19 SHMs, respectively. No meaningful correlation was observed between VH and VL mutation frequencies. The high degree of SHM of filaria-specific antibodies suggests a prolonged and ongoing humoral immune response to antigens.

Filaria-Specific IgE Antibodies Induce Anaphylaxis in Mice

We next assessed function of two filaria-specific IgE mAbs, 5H1 and 18H7, using a human FcεRIα–transgenic mouse model of passive systemic anaphylaxis. We chose these two IgE mAbs because of their ability to bind TTR protein dimers, which could in theory allow cross-linking of FcεRI to trigger mast cell degranulation. Thus, we first sensitized mice with TTR–specific IgE mAbs 5H1, 18H7, both, or with injection of an isotype control antibody. Three days later mice were challenged with single intraperitoneal injection with recombinant TTR proteins (50 μg of filarial antigen TTR_76, TTR_79, or TTR_62) or with control peanut allergen extract. All mice injected with recombinant TTRs, whether sensitized with mAbs 5H1, 18H7, or both, exhibited a significant drop in temperature of up to 6°C, reaching the nadir within 35 minutes of challenge, while animals receiving peanut allergen extract showed no temperature change (Fig. 5a, b). This response is indicative of anaphylaxis[40]. Of note, we observed no additive or synergistic effects in mice injected with both 5H1 and 18H7 IgE mAbs (Fig. 5b).

DISCUSSION

Despite our deep understanding of the major allergen proteins responsible for induction of allergic diseases, little is known about immunogenic triggers of the IgE antibody response in the defense against helminth infection. We adopted an unbiased approach to investigate specificities and functions of IgE antibodies produced in the context of filarial worm infection. Of the 56 human mAbs studied, 26 showed binding to *B. malayi* and/or *D. immitis* worm somatic extracts and none of them showed significant cross-reactivity toward commonly known allergens. We identified 14 unique filarial antigens targeted by 16 filaria-specific IgE mAbs and report here for the first time the “(TTR)-like” family of proteins as being potent filarial immunogens. It is remarkable that members of this family of proteins do not share much homology, yet of the 10 TTR-specific IgE mAbs two broadly cross-react with 13 different TTR proteins. As two thirds of our filaria-specific antibodies are targeting TTR proteins, we find this family to contain the immunodominant antigens inducing IgE antibodies in response to filarial infections. Results from our studies also show that IgE antibodies specific for filarial antigens identified here (WbSXP-1, TTRs, 24kDa secreted protein, and NPA-1) are naturally produced during infection and can be identified within the serum of subjects. These findings further confirm the unbiasedness of our study in identifying the most prominent filarial antigens and proposes a sensitive species-specific diagnostic approach.

We report that all filarial antigens targeted by IgE antibodies in our analysis were nematode-specific and E/S proteins. Our *in vivo* data shows that filaria-specific IgE antibodies induce

a robust type one hypersensitivity response. Adult filarial worms copulate within their host and produce microfilaria, but new infections are required to increase the number of adult worms. Stopping new larvae from maturing after entering the skin via mosquito bite would prevent an increase in the number of worms living within the host. Taken together, this suggests that evolutionarily the IgE antibody response developed to enable prompt recognition of invading worms at the larval stage as they penetrate the epithelial barrier. We hereby propose that IgE antibodies produced in response to parasitic infections function to prevent establishment of new infections at the epithelial site of entry. Mast cell activation occurring through IgE cross-linking associated with excreted worm antigens allows immediate detection, prompting eosinophil and basophil infiltration and targeting the worm when it is most vulnerable. This “gatekeeper” function of IgE was initially proposed and studied for its effect on microbial toxin neutralization[41-43], but also appears to be the specific role it plays in helminth immunity. Future experiments will further evaluate this role directly in defending against helminth infection by passively sensitizing hFcεRI mice with functional human IgE mAbs described here and challenging with living *B. malayi* infective stage larvae. Given the impact of helminthic diseases in developing countries, it is of central importance to identify key helminth immunogens that can inform work toward improved diagnostics and the host/parasite interface. In addition to providing insights into the ontogeny of the IgE response in helminth infection, findings reported here have implications for rational design of helminth vaccines providing IgE-mediated pathology can be circumvented.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

BmA	*Brugia malayi* somatic extracts
MAb	Monoclonal Antibody
E/S proteins	Excreted secretory proteins
LF	Lymphatic filariasis
CCD	Cross-reactive carbohydrate determinants
Th2	T-helper 2

TPE	Tropical pulmonary eosinophilia
PBMC	Peripheral blood mononuclear cells
IP	Immunoprecipitation
MS	Mass spectrometry
SHM	Somatic hypermutation
TTR	Transthyretin-related
MIF	Migration inhibitory factor
NPA	Nematode polyprotein antigen
EC50	Half maximal effective concentration
PSM	Peptide spectrum matches

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KEY MESSEGES

- Despite it becoming common knowledge that the IgE antibody response is necessary for protection from parasitic worm infections there is limited understanding of antigenic triggers underlying this response and the natural function of IgE.
- All filarial antigens targeted by IgE antibodies in our analysis were nematode-specific and excreted proteins.
- Our data suggest that IgE antibodies enable prompt immune recognition of invading worms at the barrier of entry.

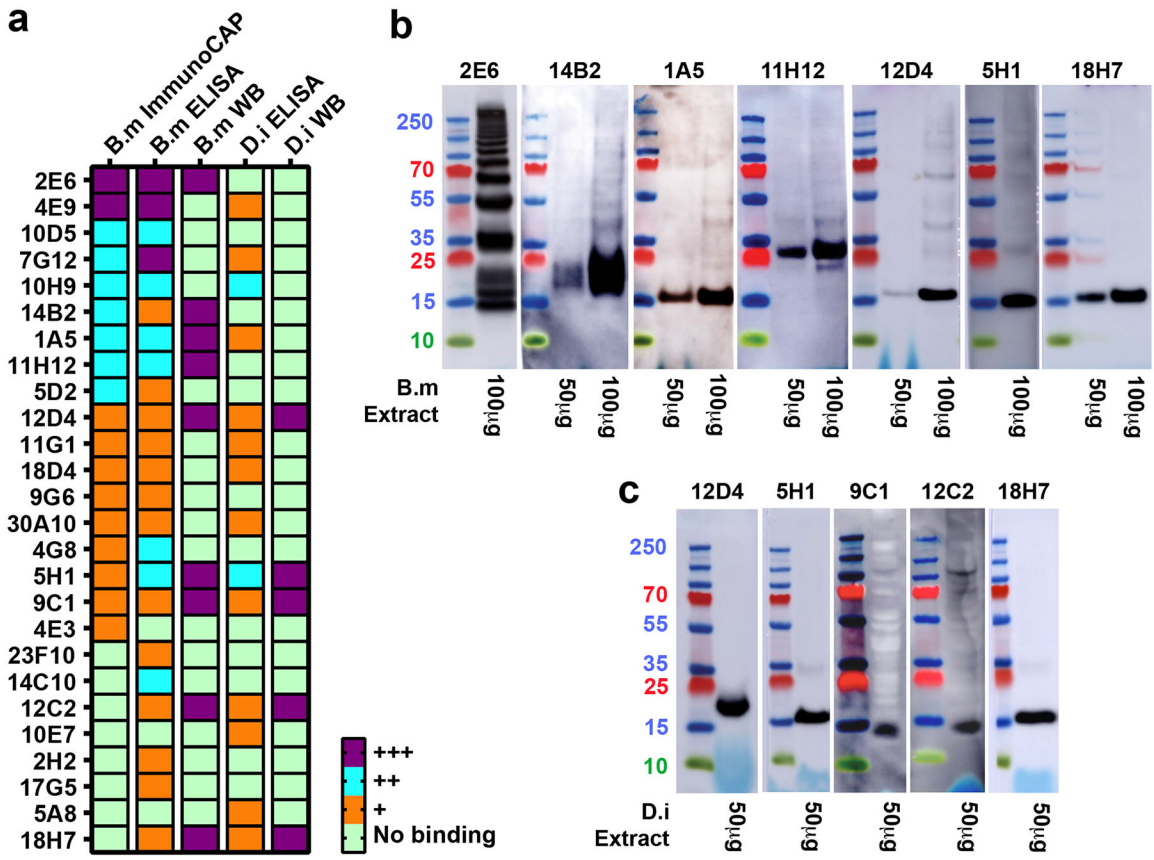

Figure 1. Characterization of IgE mAbs isolated from peripheral blood of subjects with filarial infection.

a, Heatmap representing IgE binding to *B. malayi* ImmunoCAP (considered positive if >1 kUA/L) and *B. malayi* and *D. immitis* binding in ELISA (− no binding detected; + binding 3-10 times background; ++ binding 10-100 times background; +++ binding >100 times background) and immunoblot (WB) (− no binding; +++ presence of a clear band). MAbs are ordered by ImmunoCAP binding value. Data are representative of at least two independent experiments. **b,** Immunoblot analysis of IgE mAb binding to protein in somatic extracts from *B. malayi.* **c,** Analysis as in (b) using *D. immitis* extracts. For (b) and (c) only immunoblot-positive antibodies are shown.

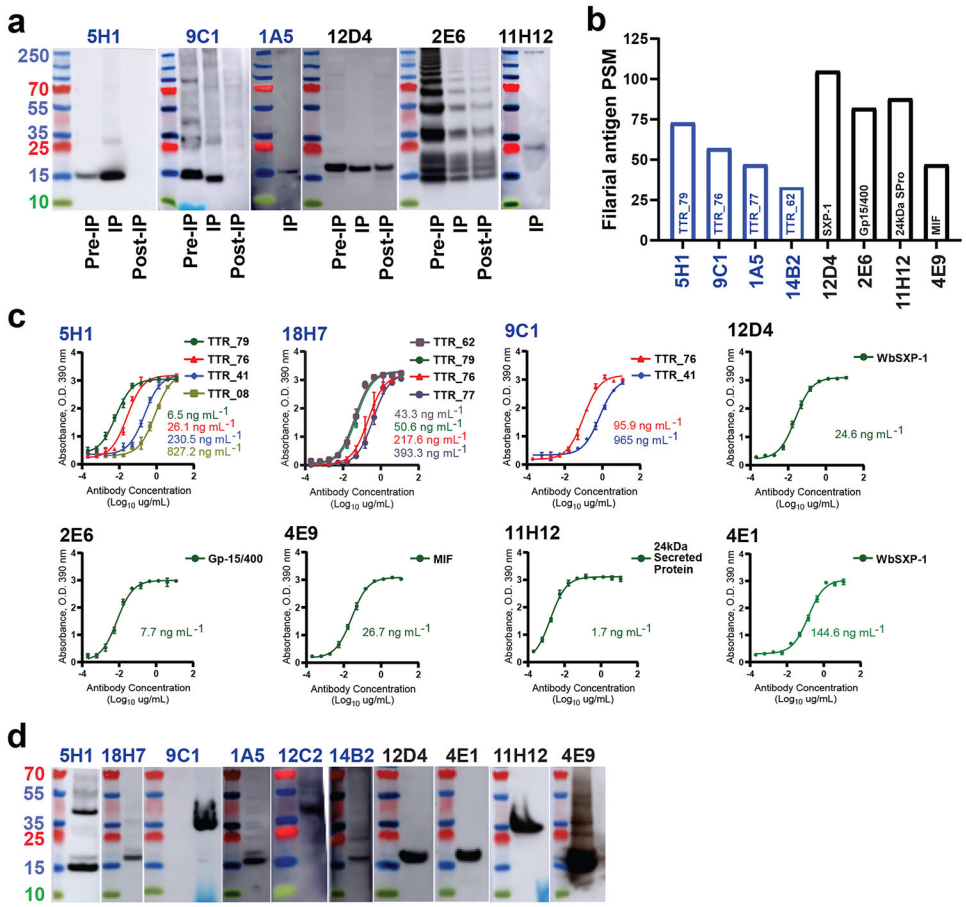

Figure 2. Identification of filarial antigens targeted by IgE mAbs.

a, Analysis of specificity of each IgE mAb for its target antigen by IP followed by immunoblot. A filarial worm somatic extract was immunoprecipitated with IgE mAbs. Whole somatic extract (Pre-IP), IP product and extract after IP (Post-IP) were then blotted with the same IgE mAb. Only immunoblot-positive antibodies are shown. **b,** Shown is the number of peptide spectrum matches (PSMs) identified for each antigen by mass spectrometry analysis. **c,** Specificity validation of each IgE mAb for its target by ELISA. Data obtained in triplicate are shown as the mean ± SEM and are representative of three experiments. Calculated EC50 values are shown on the graph. **d,** Specificity validation of each IgE mAb for its target by immunoblot analysis.

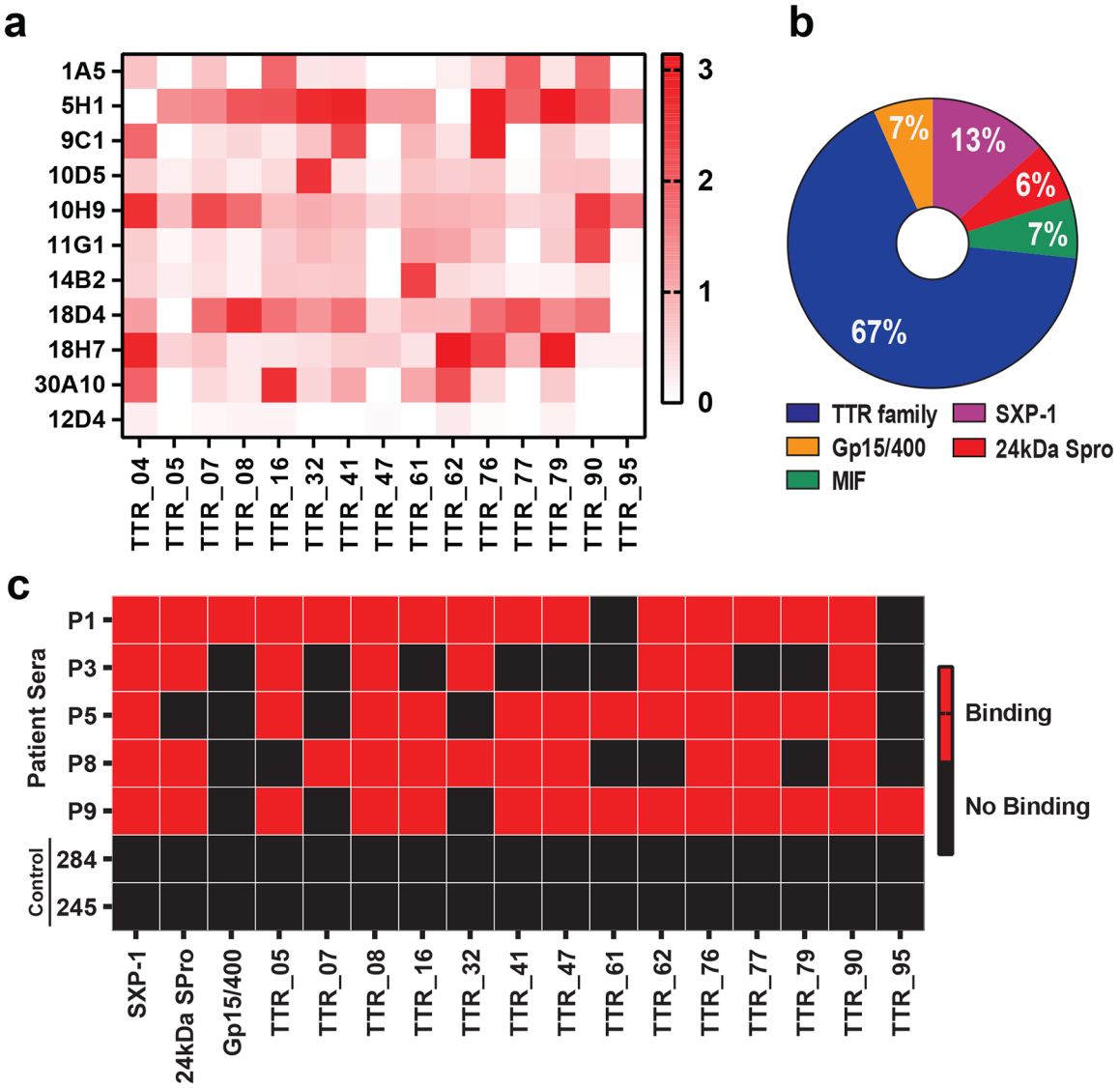

Figure 3. TTR family proteins are the prominent filarial antigens targeted by IgE antibodies and have diagnostic potential.

a, ELISA analysis depicting TTR-specific antibody cross-reactivity profile to multiple TTR proteins. MAb 12D4, a human IgE mAb specific for WbSXP-1, served as negative control. OD, optical density. **b,** Proportion of filaria-specific antibodies binding different antigens. **c,** Binary heatmap showing presence (red) or absence (black) of antibody in indicated patient sera against filarial antigens tested in immunoblot analysis. Two serum samples (284 and 245) from respective mold- and shellfish-allergic patients served as negative controls.

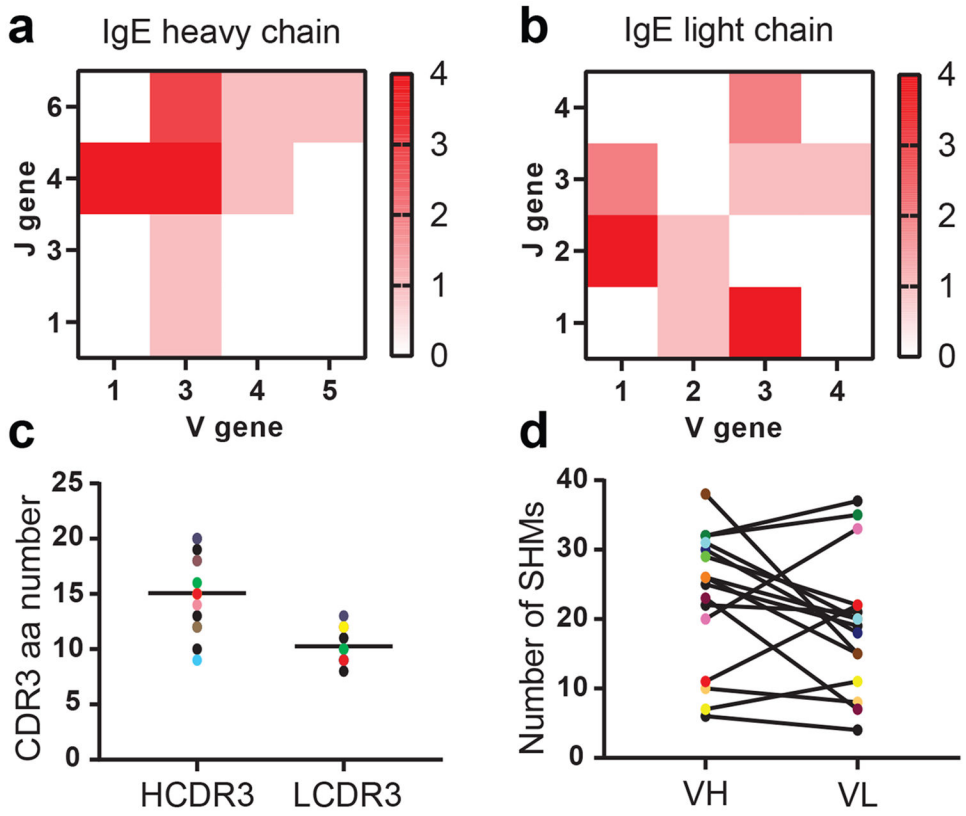

Figure 4. IgH and IgL gene sequence analysis of filaria-specific IgE antibodies.

Sixteen filaria-specific IgE antibodies were found to have unique sequences and were analyzed for genetic features. **a,** Heatmap showing the total number of unique sequences with corresponding V and J genes. **b,** CDR3 amino acid length distribution for heavy and light chains with indicated mean. **c,** IgH and IgL CDR3 amino acid number was determined using the ImMunoGeneTics (IMGT) database. **d,** Number of SHMs in the VH and VL chain of filaria-specific mAb genes.

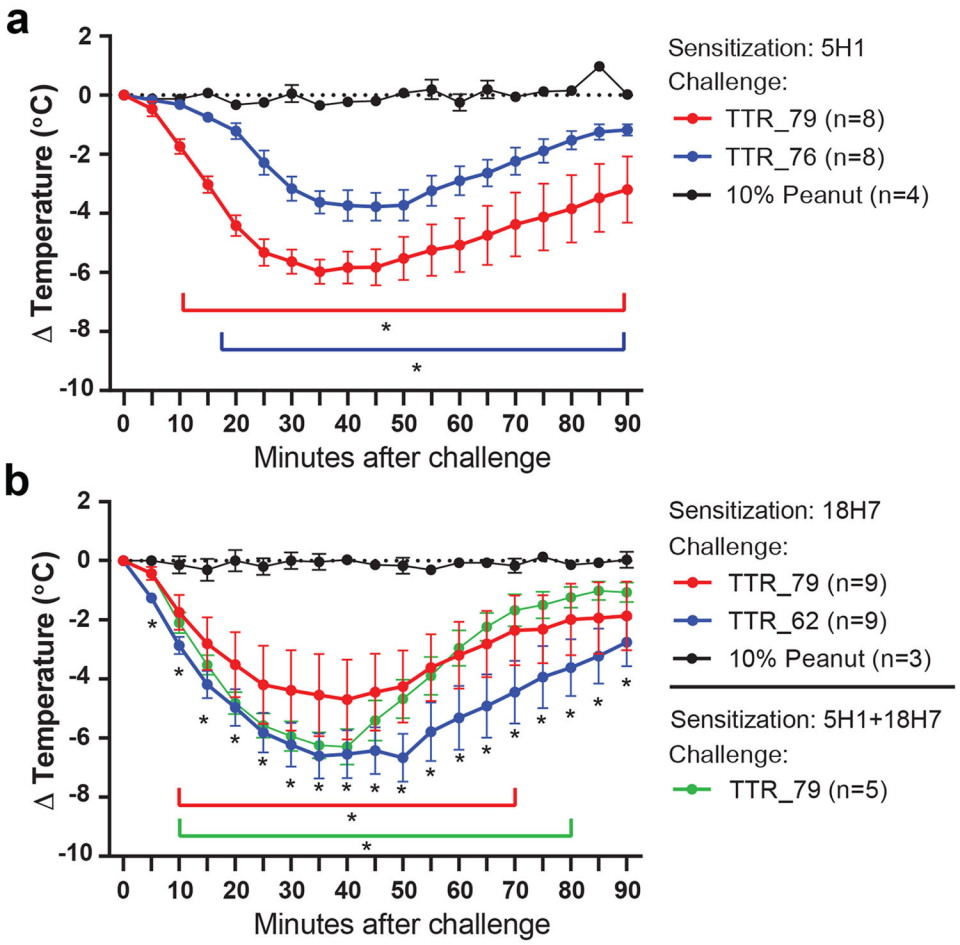

Figure 5. Filarial antigens induce anaphylaxis in IgE mAb-sensitized human FcεRIα–transgenic mice.

Mice were sensitized with a 100 μg i.p. injection of human IgE mAb. Three days later, mice were injected i.p. with 50 μg of filarial antigens or a control allergen (10% peanut extract). Body temperature over 90 minutes was monitored using an implanted temperature probe. **a,** Mice were sensitized with a 100 μg i.p. injection of human IgE mAb 5H1 and challenged with TTR_76, TTR_79, or 10% peanut extract. **b,** Mice were sensitized with 5H1 or 18H7 or both and challenged with TTR_79, TTR_62, or 10% peanut extract. Change in body temperature of experimental compared to control allergen groups at each time point using a paired 2-tailed t test assuming unequal variance. Time points with calculated P values <0.05 are highlighted by an asterisk. Data are means ± SEM of each experimental group. The number of mice (n) for each experimental group is shown.

Table 1.

Subject demographics and IgE encoding B cell frequencies

Subject code	Age	Sex	Diagnosis	Geographic location of infection	Serum IgE IU/ml	IgE B-cell frequency (per 10^7 PBMCs)	IgE hybridomas generated
P1	61	M	TPE	India	4260	13.5	22
P2	29	M	TPE	India	9810	8.1	1
P3	21	F	Loiasis	Cameroon	20290	9.2	9
P4	40	M	Loiasis	Cameroon	8520	10.9	12
P5	61	F	Loiasis	Cameroon	11362	6.2	5
P6	29	M	Loiasis	Gabon	7628	6.0	1
P7	42	M	Onchocerciasis	Sierra Leone	8680	13.7	6
P8	49	F	TPE	India	6140	Serum only	Serum only
P9	27	M	TPE	India	7440	Serum only	Serum only

Subject helminth disease, geographic location of their infection, serum IgE titer, IgE B-cell frequency and IgE hybridomas yield are shown. IgE B cell frequencies are expressed as the number of IgE-positive cells per 10 million PBMCs. The total number of IgE-expressing human hybridomas generated for each subject is listed. No PBMCs, only sera, were used from subjects P8 & P9. TPE, tropical pulmonary eosinophilia.

Table 2.

Filaria-reactive human IgE mAb target proteins

IgE mAb	Subject code	ImmunoCAP reactivity (kUA/L)	Binding to filarial worm extract		EC50 (ng/mL)	Mass spectrometry	Antigen name	Sequence ID
			ELISA	Immunoblot				
12D4	P3	5.3	+	+++	24.6	WbSXP-1	Antigen Wb14	AF063940.1
4E1	P3	0.5	−	+++	144.6	WbSXP-1	Antigen Wb14	AF063940.1
9C1	P3	1	+	+++	95.9	TTR-76	?	VDM14676.1
12C2	P3	0.3	+	+++	95.9	TTR-76	?	VDM14676 .1
5H1	P1	1.4	++	+++	6.5	TTR-79	?	EJW78979 .1
18H7	P1	0	+	+++	43.3	TTR-79, TTR-62	?	EJW86262.1, EJW78979.1
1A5	P1	14.2	++	+++	ND	TTR-77	?	VDM13477.1
10D5	P5	31	++	−	ND	TTR-32	?	VDM07632.1
11G1	P2	4.8	+	−	ND	TTR-90	?	VDM14190.1
10H9	P1	28.6	++	−	ND	TTR-04	?	EJW80404.1
14B2	P1	23.9	+	+++	ND	TTR-61	?	EJW84161.1
30A10	P4	2.5	+	−	ND	TTR-16	?	EJW86116.1
18D4	P1	3.5	+	−	ND	TTR-08	?	VDM13708.1
2E6	P1	465.7	+++	+++	7.7	Ladder protein, gp15/400	Nematode polyprotein allergen-1 (NPA-1)	AAG31482.1
4E9	P3	57.5	+++	−	26.7	Macrophage Migration Inhibitory Factor (MIF)	MIF−1	EJW88743.1
11H12	P1	12	++	+++	1.7	24kDa secreted protein	Alt-1, P22	VIO90327.1

The most prominent antigen(s) are listed for TTR-specific IgE mAbs (such as 5H1, 9C1 and 18H7) that cross-react with multiple members of the TTR family. ND: not determined due to low expression of target proteins.