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# Allergic Sensitization Underlies Hyperreactive Antigen-Specific CD4<sup>+</sup> T Cell Responses in Coincident Filarial Infection

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Among the various hypotheses put forward to explain the modulatory influence of helminth infection on allergic effector responses in humans, the IL-10-induced suppression of Th2-associated responses has been the leading candidate. To explore this helminth/allergy interaction more fully, parasite- and allergen-specific CD4<sup>+</sup> T cell responses in 12 subjects with filarial infections, and coincident allergic sensitization (filarial [Fil]<sup>+</sup>allergy [A]<sup>+</sup>) were compared with the responses to three appropriate control groups (Fil<sup>−</sup>A<sup>−</sup> [n = 13], Fil<sup>−</sup>A<sup>+</sup> [n = 12], Fil<sup>+</sup>A<sup>−</sup> [n = 11]). The most important findings revealed that Fil<sup>+</sup>A<sup>+</sup> had marked ( $p < 0.0001$  for all cytokines) increases in parasite Ag-driven Th2 (IL-4, IL-5, IL-13), Th9 (IL-9), and the regulatory (IL-10) cytokines when compared with Fil<sup>−</sup>A<sup>−</sup>. Moreover, using multiparameter flow cytometry, filarial parasite Ag induced a marked increase in not only the frequency of CD4<sup>+</sup> T cells producing IL-4, IL-5, IL-2, and TNF- $\alpha$  in Fil<sup>+</sup>A<sup>+</sup> when compared with Fil<sup>−</sup>A<sup>−</sup> patients, but also in the frequencies of polyfunctional Th2-like (CD4<sup>+</sup>IL-4<sup>+</sup>IL-5<sup>+</sup> and CD4<sup>+</sup>IL-2<sup>+</sup>IL-4<sup>+</sup>IL-5<sup>+</sup>TNF- $\alpha$ <sup>+</sup>) cells. The Th2-associated responses seen in the Fil<sup>+</sup>A<sup>+</sup> group were correlated with serum IgE levels ( $p < 0.01$ ,  $r = 0.5165$  for IL-4;  $p < 0.001$ ,  $r = 0.5544$  for IL-5; and  $p < 0.001$ ,  $r = 0.4901$  for IL-13) and levels of circulating eosinophils ( $p < 0.0116$ ,  $r = 0.5656$ ) and their degranulation/activation products (major basic protein [ $p < 0.001$ ,  $r = 0.7353$ ] and eosinophil-derived neurotoxin [ $p < 0.01$ ,  $r = 0.7059$ ]). CD4<sup>+</sup> responses to allergen were not different (to a large extent) among the groups. Taken together, our data suggest that allergic sensitization coincident with filarial infection drives parasite Ag-specific T cell hyperresponsiveness, which is characterized largely by an augmented Th2-dominated immune response. *The Journal of Immunology*, 2016, 197: 000–000.

Currently, more than three billion people worldwide are infected with helminth parasites and/or suffer from allergic diseases, including bronchial asthma, allergic rhinitis, food allergy, and eczema (1, 2). A common feature of both atopic disorders and helminth infections is their association with type 2 immune responses (3). Indeed, the canonical human immune response to filarial parasites is of the Th2 type, characterized by the production of IL-4, IL-5, IL-9, IL-10, and IL-13, induction of IgG4 and IgE Abs, as well as tissue and peripheral blood eosinophilia (4). This Th2-associated immune response has been

implicated in mediating protection to helminth infection but has also been shown to play an important role in the pathogenesis of allergic diseases (5).

The role of helminth infection in modulating allergic reactivity (a concept grouped within the “hygiene hypothesis”) (6) is based on initial studies from Gabon (7) and Ecuador (8) that demonstrated that children infected with intestinal helminths or *Schistosoma haematobium* have a lower risk of allergic sensitization (based on skin testing to aeroallergen extracts) in comparison with children without helminth parasite infections from the same geographical regions. It has been postulated that this allergen-specific immune response modulation is mediated by parasite-specific IL-10 and possibly by increases in the frequency of regulatory T cells (Tregs) (9–11). Other studies, in contrast, have indicated that infection by gastrointestinal helminths is associated with an increased incidence of allergic reactions (12–14). The explanation that perhaps allows for a reconciliation of these distinct sets of findings may rely on the relative acuteness of the helminth infection (15, 16). Indeed, given that most helminth infections are long-standing (chronic infection is the rule), it is known that the effector T cell responses (including the Th2 responses) become muted (modified Th2 response) (17), allowing for a response that balances Th2-mediated inflammation and their regulatory controls. Furthermore, it has been shown that this modulation of the Ag-specific T cell response in the context of chronic helminth infection has a propensity to induce bystander effects on responses to vaccinations, other infectious agents, and allergens (18, 19).

Thus, the present study examined the nature of the T cell response induced by parasite Ags and aeroallergens in the context of filarial-infected individuals with or without coincident allergic sensitization. Our data demonstrate that allergic sensitization coincident with filarial infection drives parasite Ag-specific T cell

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Abbreviations used in this article: A, allergy; BMA, crude extract from *Brugia malayi* adult worms; Der p, *Dermatophagoides pteronyssinus*; Fil, filarial; GM, geometric mean; kUA, kU of allergen-specific Ab; P/I, PMA/ionomycin; Treg, regulatory T cell.

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hyperresponsiveness, characterized largely by an augmented Th2-dominated immune response. These responses are associated with a marked elevation of IgE, eosinophilia, and eosinophil activation, with the latter potentially responsible for the associated clinical signs (angioedema, urticaria) often seen in these infections.

## Materials and Methods

### Study population

From a previously described cohort of 308 subjects (16, 20) used to identify crossreactive epitopes among parasite Ags and aeroallergens, 49 individuals were included in the current study population based solely on the availability of sufficient numbers of cryopreserved PBMCs. The study population is described in Table I and consisted of four study groups based on the presence or absence of atopy and/or filarial infection: group 1, filarial (Fil)<sup>+</sup>allergy (A)<sup>+</sup> ( $n = 12$ ), group 2, Fil<sup>+</sup>A<sup>-</sup> ( $n = 11$ ), group 3, Fil<sup>-</sup>A<sup>+</sup> ( $n = 13$ ), and group 4, Fil<sup>-</sup>A<sup>-</sup> ( $n = 13$ ).

All individuals (and all samples collected) were part of registered protocols approved by the Institutional Review Boards of the National Institute of Allergy and Infectious Diseases (NCT00001230 and NCT00001345) for the filarial-infected subjects and of the Department of Transfusion Medicine, Clinical Center, National Institutes of Health (IRB 99-CC-0168) for the healthy donors. Written informed consent was obtained from all subjects.

### In vitro culture

For the analysis of cytokine production by PBMCs after in vitro stimulation by parasite Ag and environmental allergens, aliquots of cell suspensions in RPMI 1640 medium supplemented with 10% human sera, 1% L-glutamine (Sigma-Aldrich), 1% antibiotic (Invitrogen), and 1% nonessentials amino acids (Sigma-Aldrich) were cultured in media alone, with crude extract from *Brugia malayi* adult worms (BMA; 10 µg/ml), with dust mite (*Dermatophagoides pteronyssinus* [Der p]) extract (ALK-Abello, Port Washington, NY; Der p extract at 15 allergen-specific units), or with PMA/ionomycin (P/I; Sigma-Aldrich) (0.5/0.05 µg/ml) for 72 h in 5% CO<sub>2</sub> at 37°C for the measurement of cytokine production. To identify the source of these cytokines by flow cytometry, PBMCs of all patient groups were cultured and stimulated under the same conditions as mentioned above, but for 12 h, with the addition of CD28/CD45d costimulatory molecules (BD FastImmune; BD Biosciences) and 10 µg/ml brefeldin A/monensin (Sigma-Aldrich).

### Luminex assay

The quantification of IL-2, IL-4, IL-5, IL-9, IL-10, IL-13, IL-17A, IL-22, TNF-α, and IFN-γ levels was assessed by a Luminex multiplex assay (Milliplex; Millipore) in accordance with the manufacturer's recommendations. The eosinophil granule proteins were measured using a previously described assay (21).

### Multiparameter flow cytometry

To identify the source of the Th1/Th2/Th9/Th17-associated and regulatory cytokines, cells from in vitro cultures were harvested and washed with FACS buffer (PBS, 2% FBS, and 0.1% sodium azide) and then incubated with Fc blocking solution for 20 min. The cells were then stained for viability (Live/Dead fixable blue, Molecular Probes), washed with FACS buffer, and then incubated with fluorochrome-conjugated anti-CD3, anti-CD4, and anti-CD8 for 30 min in the dark at room temperature. The cells were next washed twice with FACS buffer, then fixed and permeabilized using a Fix/Perm buffer kit (BioLegend) for 20 min in the dark at 4°C. The cells were washed twice with Perm buffer (BioLegend) and resuspended with the fluorochrome-conjugated anti-IL-2, anti-IL-4, anti-IL-5, anti-IL-10, anti-IL-17A, anti-TNF-α, anti-IFN-γ, and anti-CD25 pool (Supplemental Table I) for 30 min in the dark at 4°C. Finally, the cells were washed twice with Perm buffer and then acquired using the BD LSRFortessa flow cytometer (BD Biosciences) and FACSDiva software (BD Biosciences) for acquisition. All analyses were performed using FlowJo v10.0.8. For the flow cytometry multiparameter analyses, PESTLE/SPICE v5.0 (National Institute of Allergy and Infectious Diseases) software was used. Additionally, the frequency of CD4<sup>+</sup> T cells producing single and multiple cytokines was analyzed by the FlowJo software based on the gating strategy shown in Supplemental Fig. 1.

### Data analysis

All statistical analyses were performed using Prism 5.0 for Windows (GraphPad Software). Unless stated otherwise, geometric means (GMs) and

the upper 95% confidence interval of GM (between parentheses) were used as measures of central tendency. The D'Agostino and Pearce normality test was used to determine whether data sets were normally distributed. A Kruskal-Wallis test followed by Dunn multiple comparison tests were used to evaluate the statistical differences in the cytokine production by the different groups, as well as the frequency of CD4<sup>+</sup> T cells producing cytokines. Correlations were performed using the Spearman rank correlation. Net production of cytokines (in picograms per milliliter) and net frequency of CD4<sup>+</sup> T cell-producing cytokines (in percentage) were calculated by subtracting the baseline level from the level following stimulation. The differences were considered statistically significant when the corrected  $p$  values were <0.05.

## Results

### Study population

The clinical diagnoses, IgE (total and allergen-specific) levels, and peripheral eosinophil counts for the study population can be found in Table I. Peripheral blood eosinophil counts were greater in the Fil<sup>+</sup>A<sup>+</sup> patients compared with those filarial-infected patients without allergy (Fil<sup>+</sup>A<sup>-</sup>) (GM = 2269 [4449.0] versus 590 [1312.0] cells/µl;  $p = 0.0151$ ). Interestingly, the Fil<sup>+</sup>A<sup>+</sup> patients also demonstrated a significant increase in the levels of IgE (GM = 1644 [3865] kU of allergen-specific Abs [kUA]/l) when compared with Fil<sup>+</sup>A<sup>-</sup> patients (GM = 122 [323.1] kUA/l;  $p < 0.05$ ), Fil<sup>-</sup>A<sup>+</sup> individuals (GM = 84.0 [168.1] kUA/l;  $p < 0.05$ ), and Fil<sup>-</sup>A<sup>-</sup> healthy donors (GM = 9.0 [19.74] kUA/l;  $p < 0.001$ ).

### Environmental allergy induces a hyperresponsiveness in the concomitant immune response to filarial infection mediated by parasite Ags

To elucidate the influence of atopy on the parasite- and allergen-induced immune responses, we first assessed cytokine production. As shown in Fig. 1, when spontaneous production of the various Th2-associated cytokines was measured (in the absence of a stimulus), there were no differences among the four groups in any of the cytokines measured (Fig. 1A–E). In marked contrast, when stimulated with the filarial parasite Ag BMA, those filarial-infected individuals with concomitant atopy (Fil<sup>+</sup>A<sup>+</sup>) had marked and significant increases in the levels of IL-5 (GM = 232.1 [504.3] versus 4.9 [38.34] pg/ml;  $p < 0.05$ ), IL-13 (GM = 826.9 [1732] versus 14.9 [175.6] pg/ml;  $p < 0.05$ ), IL-9 (GM = 83.1 [178.8] versus 2.8 [24.69] pg/ml;  $p < 0.05$ ), and IL-10 (GM = 36.1 [65.45] versus 9.6 [52.82] pg/ml;  $p < 0.05$ ) when compared with Fil<sup>+</sup>A<sup>-</sup>. These differences were even more pronounced when the IL-4 (GM = 0.1 [0.24] pg/ml,  $p < 0.01$  and GM = 0.1 [0.21] pg/ml,  $p < 0.01$ ), IL-5 (GM = 0.7 [2.98] pg/ml,  $p < 0.001$  and GM = 0.7 [2.89] pg/ml,  $p < 0.001$ ), IL-13 (GM = 6.3 [35.66] pg/ml,  $p < 0.001$  and GM = 4.2 [32.76] pg/ml,  $p < 0.001$ ), IL-9 (GM = 0.6 [1.99] pg/ml,  $p < 0.001$  and GM = 0.5 [1.82] pg/ml,  $p < 0.001$ ), and IL-10 (GM = 0.5 [1.56] pg/ml,  $p < 0.001$  and GM = 0.2 [0.72] pg/ml,  $p < 0.001$ ) responses were compared with the other two groups (Fil<sup>-</sup>A<sup>+</sup> and Fil<sup>-</sup>A<sup>-</sup>, respectively Fig. 1F–J). No relevant differences were observed among the groups in the cytokine production after Der p allergen stimulation (Fig. 1K–O). Cytokine responses following P/I stimulation showed that Fil<sup>+</sup>A<sup>+</sup> had a marked increase in the levels of IL-4 ( $p < 0.01$ ), IL-5 ( $p < 0.001$ ), and IL-9 ( $p < 0.001$ ) when compared with Fil<sup>-</sup>A<sup>-</sup> (Fig. 1P, 1Q, 1S). When levels of IL-13 were evaluated, the Fil<sup>+</sup>A<sup>+</sup> had levels of IL-13 that were strikingly more pronounced than those seen in comparison with Fil<sup>+</sup>A<sup>-</sup> ( $p < 0.05$ ) and Fil<sup>-</sup>A<sup>-</sup> ( $p < 0.001$ ) (Fig. 1R). No differences were seen among the groups for P/I-driven IL-10. The analysis of spontaneous production and BMA, Der p allergen, and P/I-driven type 1- and type 17-associated cytokines showed no differences in the production of IL-2, TNF-α, IFN-γ, IL-17, and IL-22 production among the four groups (Supplemental Fig. 2).

Table I. Description of the study population

| Group                           | ID | Diagnosis                   | Phadiatop (kUA/L) <sup>a</sup> | Der p-Specific IgE (kUA/L) | Total IgE (kUA/L) | Eosinophils (cells/mm <sup>3</sup> ) |
|---------------------------------|----|-----------------------------|--------------------------------|----------------------------|-------------------|--------------------------------------|
| Fil <sup>+</sup> A <sup>+</sup> | 1  | <i>Loa loa</i>              | 22.5                           | 11.8                       | 497.0             | 400                                  |
| Fil <sup>+</sup> A <sup>+</sup> | 2  | <i>Wuchereria bancrofti</i> | 5.4                            | 13.3                       | 120.0             | 427                                  |
| Fil <sup>+</sup> A <sup>+</sup> | 3  | <i>O. volvulus</i>          | 48.0                           | 16.1                       | 755.0             | 3,080                                |
| Fil <sup>+</sup> A <sup>+</sup> | 4  | <i>L. loa</i>               | 43.4                           | 38.5                       | 1,244.0           | 7,520                                |
| Fil <sup>+</sup> A <sup>+</sup> | 5  | <i>L. loa</i>               | 5.3                            | 4.8                        | 9,296.0           | 1,408                                |
| Fil <sup>+</sup> A <sup>+</sup> | 6  | <i>L. loa</i>               | 26.4                           | 2.3                        | 11,794.0          | 10,414                               |
| Fil <sup>+</sup> A <sup>+</sup> | 7  | <i>L. loa</i>               | 30.8                           | 45.7                       | 1,269.0           | 7,283                                |
| Fil <sup>+</sup> A <sup>+</sup> | 8  | <i>L. loa</i>               | 4.1                            | 3.2                        | 6,206.0           | 1,404                                |
| Fil <sup>+</sup> A <sup>+</sup> | 9  | <i>L. loa</i>               | 1.4                            | 0.6                        | 606.0             | 4,218                                |
| Fil <sup>+</sup> A <sup>+</sup> | 10 | <i>L. loa</i>               | 35.7                           | 2.1                        | 1,774.0           | 3,267                                |
| Fil <sup>+</sup> A <sup>+</sup> | 11 | <i>L. loa</i>               | 1.4                            | 0.7                        | 1,506.0           | 1,709                                |
| Fil <sup>+</sup> A <sup>+</sup> | 12 | <i>L. loa</i>               | 3.5                            | 3.3                        | 4,969.0           | 1,334                                |
| Fil <sup>+</sup> A <sup>-</sup> | 1  | <i>L. loa</i>               | 0.0                            | —                          | 43.3              | 1,240                                |
| Fil <sup>+</sup> A <sup>-</sup> | 2  | <i>L. loa</i>               | 0.0                            | —                          | 115.0             | 560                                  |
| Fil <sup>+</sup> A <sup>-</sup> | 3  | <i>L. loa</i>               | 0.0                            | —                          | 187.0             | 141                                  |
| Fil <sup>+</sup> A <sup>-</sup> | 4  | <i>L. loa</i>               | 0.0                            | —                          | 33.9              | 4,104                                |
| Fil <sup>+</sup> A <sup>-</sup> | 5  | <i>L. loa</i>               | 0.0                            | —                          | 2,794.0           | 675                                  |
| Fil <sup>+</sup> A <sup>-</sup> | 6  | <i>L. loa</i>               | 0.0                            | —                          | 106.0             | 1,650                                |
| Fil <sup>+</sup> A <sup>-</sup> | 7  | <i>O. volvulus</i>          | 0.0                            | —                          | 172.0             | 252                                  |
| Fil <sup>+</sup> A <sup>-</sup> | 8  | <i>W. bancrofti</i>         | 0.0                            | —                          | 99.2              | 88                                   |
| Fil <sup>+</sup> A <sup>-</sup> | 9  | <i>L. loa</i>               | 0.0                            | —                          | 787.0             | 1,580                                |
| Fil <sup>+</sup> A <sup>-</sup> | 10 | <i>W. bancrofti</i>         | 0.0                            | —                          | 26.0              | 200                                  |
| Fil <sup>+</sup> A <sup>-</sup> | 11 | <i>L. loa</i>               | 0.0                            | —                          | 28.0              | 960                                  |
| Fil <sup>-</sup> A <sup>+</sup> | 1  | Negative                    | 21.9                           | 3.5                        | 457.0             | —                                    |
| Fil <sup>-</sup> A <sup>+</sup> | 2  | Negative                    | 5.6                            | 2.6                        | 107.0             | —                                    |
| Fil <sup>-</sup> A <sup>+</sup> | 3  | Negative                    | 11.9                           | 8.7                        | 43.8              | —                                    |
| Fil <sup>-</sup> A <sup>+</sup> | 4  | Negative                    | 11.1                           | 0.8                        | 35.2              | —                                    |
| Fil <sup>-</sup> A <sup>+</sup> | 5  | Negative                    | 24.1                           | 5.7                        | 74.3              | —                                    |
| Fil <sup>-</sup> A <sup>+</sup> | 6  | Negative                    | 13.7                           | 1.2                        | 283.0             | —                                    |
| Fil <sup>-</sup> A <sup>+</sup> | 7  | Negative                    | 7.7                            | 6.8                        | 10.7              | —                                    |
| Fil <sup>-</sup> A <sup>+</sup> | 8  | Negative                    | 26.6                           | 8.1                        | 60.1              | —                                    |
| Fil <sup>-</sup> A <sup>+</sup> | 9  | Negative                    | 5.6                            | 2.6                        | 18.4              | —                                    |
| Fil <sup>-</sup> A <sup>+</sup> | 10 | Negative                    | 2.7                            | 2.0                        | 63.2              | —                                    |
| Fil <sup>-</sup> A <sup>+</sup> | 11 | Negative                    | 14.5                           | 2.1                        | 121.8             | —                                    |
| Fil <sup>-</sup> A <sup>+</sup> | 12 | Negative                    | 56.0                           | 0.4                        | 399.3             | —                                    |
| Fil <sup>-</sup> A <sup>+</sup> | 13 | Negative                    | 39.8                           | 9.4                        | 191.1             | —                                    |
| Fil <sup>-</sup> A <sup>-</sup> | 1  | Negative                    | 0.0                            | —                          | 5.6               | —                                    |
| Fil <sup>-</sup> A <sup>-</sup> | 2  | Negative                    | 0.0                            | —                          | 8.4               | —                                    |
| Fil <sup>-</sup> A <sup>-</sup> | 3  | Negative                    | 0.0                            | —                          | 2.4               | —                                    |
| Fil <sup>-</sup> A <sup>-</sup> | 4  | Negative                    | 0.0                            | —                          | 9.7               | —                                    |
| Fil <sup>-</sup> A <sup>-</sup> | 5  | Negative                    | 0.0                            | —                          | 8.9               | —                                    |
| Fil <sup>-</sup> A <sup>-</sup> | 6  | Negative                    | 0.0                            | —                          | 24.3              | —                                    |
| Fil <sup>-</sup> A <sup>-</sup> | 7  | Negative                    | 0.0                            | —                          | 2.0               | —                                    |
| Fil <sup>-</sup> A <sup>-</sup> | 8  | Negative                    | 0.0                            | —                          | 143.0             | —                                    |
| Fil <sup>-</sup> A <sup>-</sup> | 9  | Negative                    | 0.0                            | —                          | 7.1               | —                                    |
| Fil <sup>-</sup> A <sup>-</sup> | 10 | Negative                    | 0.0                            | —                          | 81.1              | —                                    |
| Fil <sup>-</sup> A <sup>-</sup> | 11 | Negative                    | 0.0                            | —                          | 2.6               | —                                    |
| Fil <sup>-</sup> A <sup>-</sup> | 12 | Negative                    | 0.0                            | —                          | 3.1               | —                                    |
| Fil <sup>-</sup> A <sup>-</sup> | 13 | Negative                    | 0.0                            | —                          | 7.7               | —                                    |

<sup>a</sup>Allergen-specific IgE levels >0.35 kUA/L were considered allergic/atopic.

When the frequencies of cytokine-producing CD4<sup>+</sup> T cells were assessed ex vivo and in response to antigenic and mitogenic stimuli, a unique pattern of immune response was seen in those filarial-infected individuals with concomitant atopy (Fil<sup>+</sup>A<sup>+</sup>). In the ex vivo analyses, there were no significant differences among the four groups in the frequency of T cells producing a given cytokine in the absence of a stimulus (Supplemental Fig. 3). However, when stimulated with the filarial parasite Ag BMA (Fig. 2), Fil<sup>+</sup>A<sup>+</sup> patients demonstrated a marked increase in the frequency of CD4<sup>+</sup> T cells producing IL-4 ( $p < 0.001$ ; Fig. 2A), IL-5 ( $p < 0.05$ ; Fig. 2B), IL-2 ( $p < 0.05$ ; Fig. 2D), and TNF- $\alpha$  ( $p < 0.05$ ; Fig. 2F) when compared with the Fil<sup>+</sup>A<sup>-</sup> group. There was an even more marked disparity in the responses when the Fil<sup>+</sup>A<sup>+</sup> group was compared with the other two groups (Fil<sup>-</sup>A<sup>-</sup>, Fil<sup>-</sup>A<sup>+</sup>) ( $p < 0.01$  for each comparison). Moreover, when the expression of CD25 was measured on the CD4<sup>+</sup> T cells of the four groups (to assess Ag-specific T cell activation), it was clear that

BMA induced a marked increase in the per cell surface expression of CD25 in the Fil<sup>+</sup>A<sup>+</sup> group, both in comparison with the baseline of the same group ( $p < 0.001$ ) and when compared with the BMA stimulation in the other three groups (Fil<sup>+</sup>A<sup>-</sup>,  $p < 0.05$ ; Fil<sup>-</sup>A<sup>+</sup>,  $p < 0.05$ ; Fil<sup>-</sup>A<sup>-</sup>,  $p < 0.001$ ) (Fig. 2H). No differences were observed after Der p allergen stimulation (Supplemental Fig. 3) or after P/I stimulation, confirming the relative Ag specificity of the hyperreactive CD4<sup>+</sup> T cell response in the Fil<sup>+</sup>A<sup>+</sup> group (Supplemental Fig. 3).

Having demonstrated a marked increase in CD4<sup>+</sup> T cells producing Th1- or Th2-associated cytokines in Fil<sup>+</sup>A<sup>+</sup> individuals, we performed multiparameter analysis (Fig. 3) on the CD4 response in all groups. As shown in Fig. 3A–C there were no differences among the groups in the spontaneous/homeostatic frequencies of CD4<sup>+</sup>IL-2<sup>+</sup>TNF- $\alpha$ <sup>+</sup> cells, CD4<sup>+</sup>IL-4<sup>+</sup>IL-5<sup>+</sup> cells, and CD4<sup>+</sup>IL-2<sup>+</sup>IL-4<sup>+</sup>IL-5<sup>+</sup> TNF- $\alpha$  cells. However, in response to the filarial Ag BMA, those in the Fil<sup>+</sup>A<sup>+</sup> group had a marked increase in the

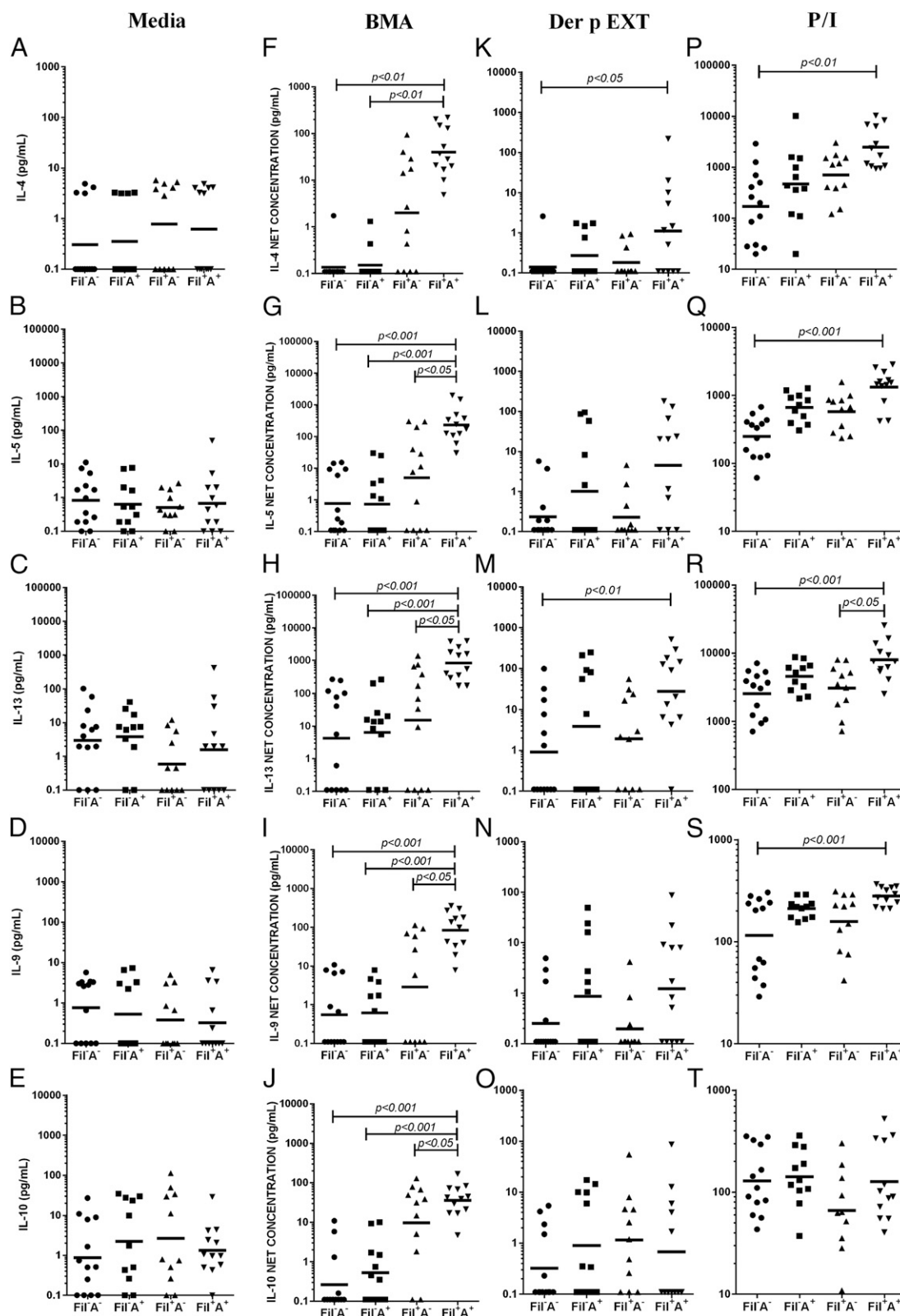


**FIGURE 1.** Th2-associated cytokines driven by parasite Ags, allergens, and mitogen in the filarial-infected individuals with or without coincident allergic sensitization. In vitro production of IL-4 (A, F, K, and P), IL-5 (B, G, L, and Q), IL-13 (C, H, M, and R), IL-9 (D, I, N, and S), and IL-10 (E, J, O, and T) at baseline (A–E) and after stimulation by filaria Ag (F–J), Der p extract (K–O), and P/I (P–T) in the four groups (FilA<sup>-</sup>, FilA<sup>+</sup>, FilA<sup>-</sup>, FilA<sup>+</sup>). Each dot represents a single individual and the horizontal bars are the GMs. Net production of cytokines (in picograms per milliliter) calculated by subtracting the baseline level from the level following stimulation.

frequency of CD4<sup>+</sup>IL-4<sup>+</sup>IL-5<sup>+</sup> T cells (GM = 0.053 [0.01]%), when compared with FilA<sup>+</sup> (GM = 0.004 [0.01]%,  $p < 0.05$ ), FilA<sup>-</sup> (GM = 0.003 [0.007]%,  $p < 0.001$ ), and FilA<sup>-</sup> (GM = 0.001

[0.001]%,  $p < 0.001$ ) (Fig. 3E). Interestingly, the FilA<sup>+</sup> group also had a marked increase in the frequency of the relatively infrequent polyfunctional CD4<sup>+</sup> subset CD4<sup>+</sup>IL-2<sup>+</sup>IL-4<sup>+</sup>IL-5<sup>+</sup>TNF- $\alpha$ <sup>+</sup> when

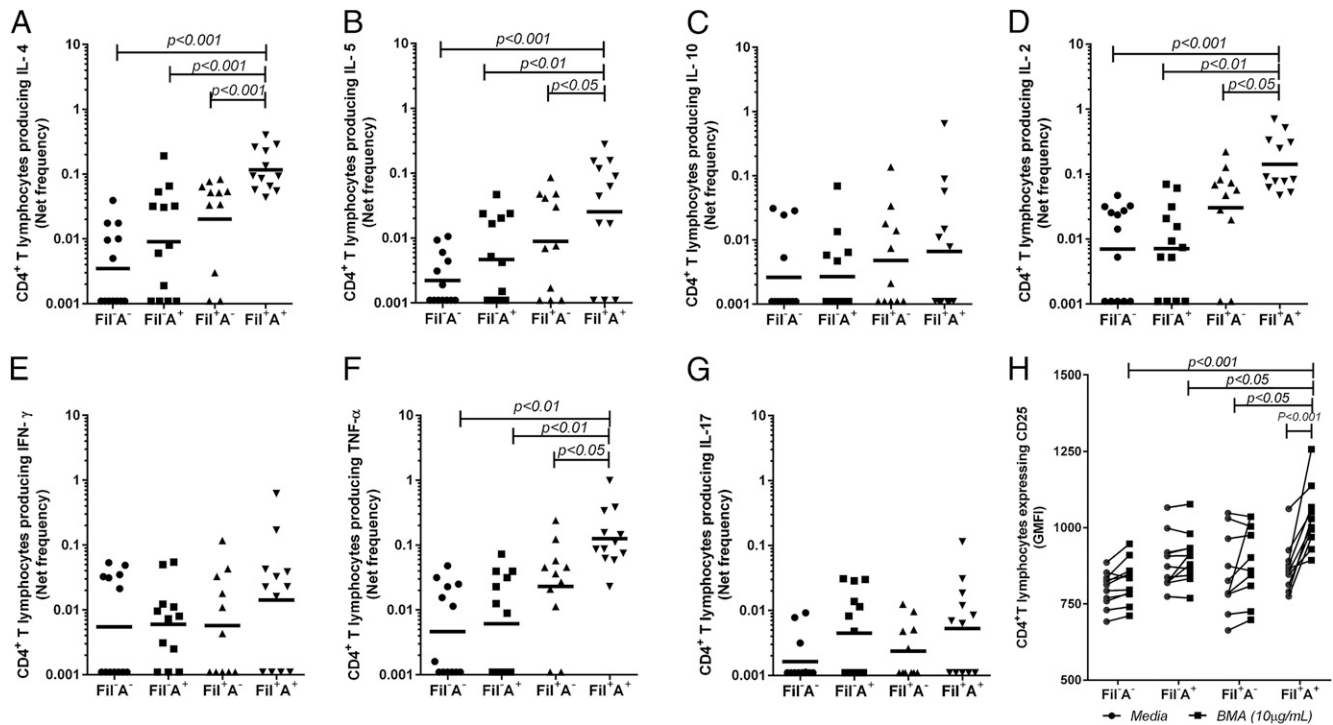


**FIGURE 2.** Ag-specific CD4<sup>+</sup> T cells hyperresponsiveness in the filarial-infected individuals with or without coincident allergic sensitization. Frequency of CD4<sup>+</sup> T lymphocytes of the four groups, producing IL-4 (**A**), IL-5 (**B**), IL-10 (**C**), IL-2 (**D**), IFN- $\gamma$  (**E**), TNF- $\alpha$  (**F**), and IL-17A (**G**) and expressing surface CD25 (**H**) after stimulation with filarial parasite Ag (BMA, 10  $\mu$ g/ml). Each dot represents a single individual, and the horizontal bars are GMs.

compared with Fil<sup>+</sup>A<sup>-</sup> patients (GM = 0.018 [0.043] versus 0.002 [0.004]%,  $p < 0.05$ ) (Fig. 3F). There were no differences among the groups when multiparameter flow cytometric analyses were examined in response to dust mite extract.

*The polarized Th2 response mediated by filarial parasite Ag may be responsible for sustaining the elevated levels of blood eosinophils and IgE in the Fil<sup>+</sup>A<sup>+</sup> patients*

To explore the relationship between the parasite-induced T cell responses and the regulation of eosinophilia, eosinophil activation, and IgE, we examined the interrelationships among these various parameters (Fig. 4). As shown in Fig. 4A, the Fil<sup>+</sup>A<sup>+</sup> group had a distinctly increased number of circulating peripheral eosinophils compared with Fil<sup>+</sup>A<sup>-</sup> patients ( $p < 0.05$ ), and those cells were strongly and positively correlated with the IL-5 levels driven by BMA Ag ( $p = 0.005$  and  $r = 0.560$ ) (Fig. 4B). Although increases in the number of peripheral blood eosinophils are often seen in helminth infections (and some allergic disorders as well), it is the activation of these eosinophils that often drives the eosinophil-mediated pathology, activation that is reflected in the serum levels of eosinophil-specific granule proteins (major basic protein, eosinophil-derived neurotoxin, eosinophil cationic protein, and eosinophil peroxidase). As shown in Fig. 4C and 4D, there was an increase in the levels of major basic protein ( $p < 0.01$  and  $p < 0.01$ ; Fig. 4C) and eosinophil-derived neurotoxin ( $p < 0.0001$  and  $p < 0.001$ ; Fig. 4D) in the Fil<sup>+</sup>A<sup>+</sup> patients in comparison with Fil<sup>+</sup>A<sup>-</sup> and Fil<sup>-</sup>A<sup>-</sup> groups, respectively. There was also a clear relationship between the levels of these granule proteins and the number of circulating eosinophils ( $p = 0.0017$  and  $r = 0.7353$ ;  $p = 0.0030$  and  $r = 0.7059$ ; Fig. 4E). Not unlike what was seen for eosinophil levels, Fil<sup>+</sup>A<sup>+</sup> individuals demonstrated significantly increased levels of IgE when compared with Fil<sup>+</sup>A<sup>-</sup> ( $p < 0.05$ ), Fil<sup>-</sup>A<sup>+</sup> ( $p < 0.05$ ), and Fil<sup>-</sup>A<sup>-</sup> ( $p < 0.001$ ) groups. Moreover, the IgE was shown to be strongly correlated with the levels of IL-4 ( $p = 0.0002$  and  $r = 0.5165$ ; Fig. 4G), IL-5 ( $p < 0.001$  and

$r = 0.5544$ ; Fig. 4H), and IL-13 ( $p < 0.0004$  and  $r = 0.4901$ ; Fig. 4I) driven by BMA.

## Discussion

Put into the context of the hygiene hypothesis, it is thought that the lack of exposure by children early in their development to microbes/parasites (as seen in most high- and middle-income countries) may explain the increased incidence of allergic diseases (6, 22) seen in these same countries, providing a causal link between helminth infection and protection from allergy. However, a wide range of studies in humans and model systems have provided substantive but conflicting evidence of the relationship between helminths and allergy, with different studies providing evidence of a positive association (23, 24), a negative association (25), or no association at all (26). This lack of consensus likely reflects real differences among the studies if the modulatory effects of helminth infections on allergic reactivity differ either because of species differences among particular helminths or because of differences in the timing of parasite infection in relationship to immune maturation or sensitization (27).

Although initial exposures to helminths may be associated with enhanced allergic inflammatory responses to the parasite, in long-term infections and with repeated infections, the host inflammatory response becomes more tightly controlled (28, 29). Chronic helminth infections induce potent immunoregulatory pathways (30–32), such as immunosuppressive cytokines (e.g., IL-10 or TGF- $\beta$ ) (33, 34) or Treg populations (35, 36) that may facilitate parasite survival. This regulation, however, may not just affect responses to parasite Ags but also to bystander Ags and aeroallergens. Such helminth-associated regulatory effects may contribute to the decreased prevalence of allergic diseases (29, 37) reported from the rural tropics.

The present study was designed to understand the mechanisms underlying the parasite-driven CD4<sup>+</sup> T cell response and the effect of coincident allergic sensitization on this process. The most important findings revealed that filarial-infected patients with coincident atopy had marked increases in parasite Ag-driven Th2

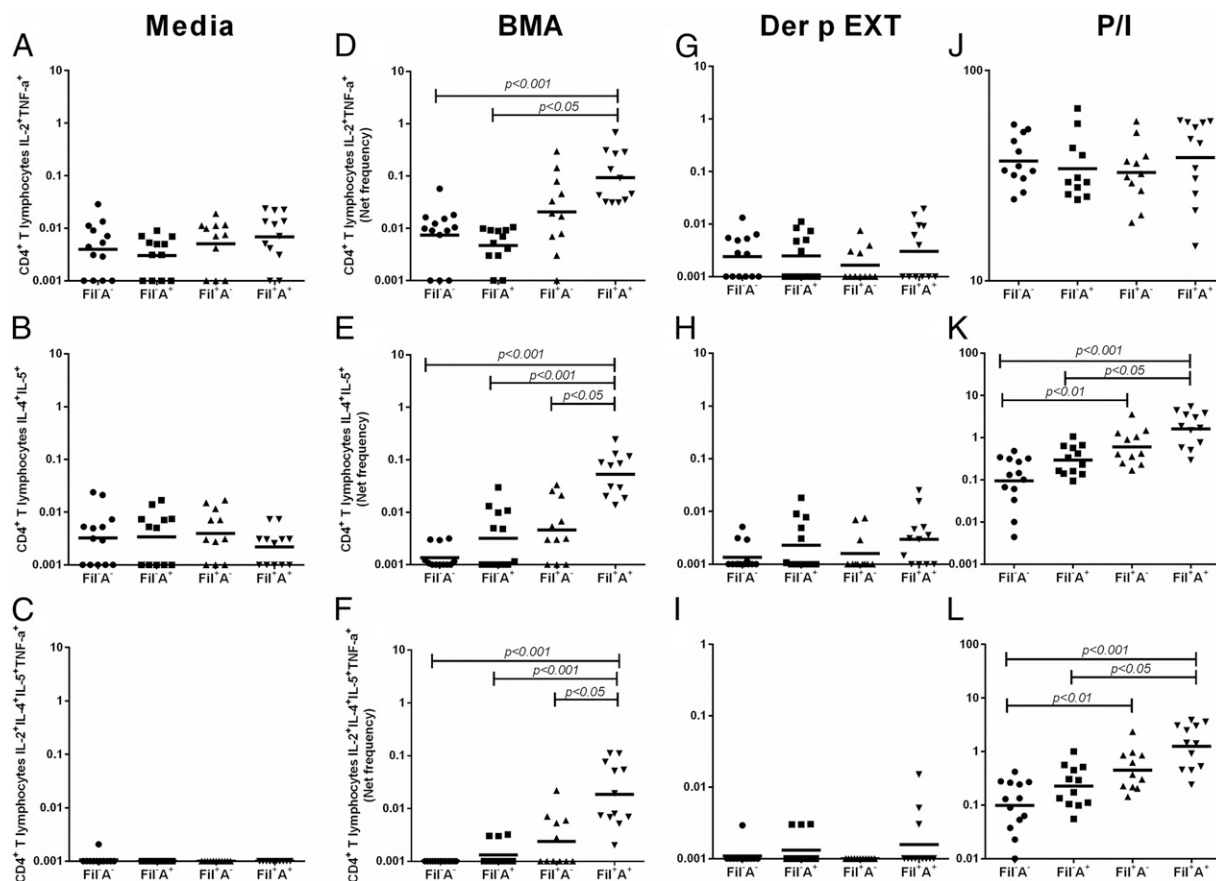


**FIGURE 3.** Flow cytometry multiparameter analysis highlighting the increased frequency of polyfunctional CD4<sup>+</sup> T cell subsets. Frequency of CD4<sup>+</sup> T lymphocytes producing multiple Th1 (**A**, **D**, **G**, and **J**), Th2 (**B**, **E**, **H**, and **K**), and mixed Th1/Th2 (**C**, **F**, **I**, and **L**) at baseline (**A**, **B**, and **C**) and after stimulation with the filarial Ag (BMA) (**D**, **E**, and **F**), Der p EXT (**G**, **H**, and **I**), and P/I (**J**, **K**, and **L**) is shown. Each dot represents a single individual, and the horizontal bars are GMs.

(IL-4, IL-5, IL-13) and Th9 (IL-9) cytokines and adaptive Tregs (IL-10) when compared with filarial-infected but nonallergic patients. Moreover, filarial parasite Ag induced a marked increase in the frequency of CD4<sup>+</sup> T cells producing IL-4, IL-5, IL-2, and

TNF-α in Fil<sup>+</sup>A<sup>+</sup> when compared with Fil<sup>+</sup>A<sup>-</sup> patients, as well as a dominant Th2 cell expansion, characterized by a marked increase in the frequencies of polyfunctional (CD4<sup>+</sup>IL-4<sup>+</sup>IL-5<sup>+</sup> and CD4<sup>+</sup>IL-2<sup>+</sup>IL-4<sup>+</sup>IL-5<sup>+</sup>TNF-α<sup>+</sup>) cells in the Fil<sup>+</sup>A<sup>+</sup> group when

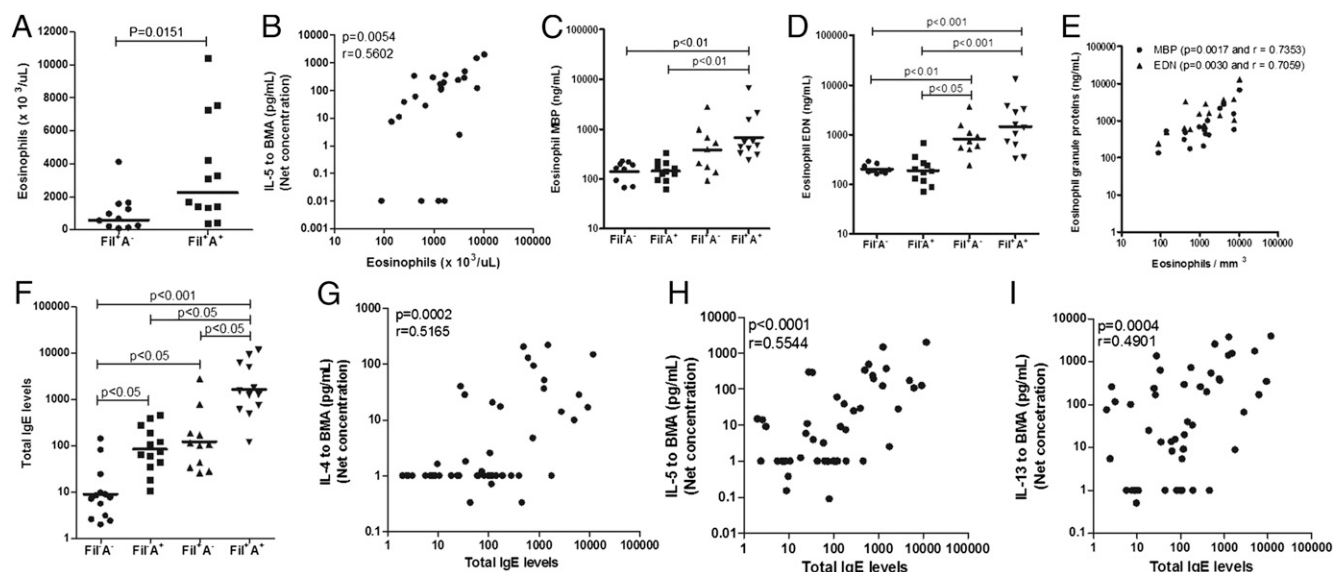


**FIGURE 4.** Eosinophilia and IgE regulation in the four groups. Eosinophils counts (**A**) and their association with BMA-specific IL-5 (**B**), eosinophil granule proteins (**C** and **D**) and their correlation with eosinophils (**E**), and IgE levels (**F**) and their association with BMA-specific Th2 cytokines (**G-I**) are shown. Each dot represents a single individual, and the horizontal bars are GMs. EDN, eosinophil-derived neurotoxin; MBP, major basic protein.

compared with  $\text{Fil}^+\text{A}^-$  patients. Furthermore, we found that this increase in Th2-associated cytokine production was reflected in IgE serum levels, increases in circulating eosinophils, and measurements of eosinophil activation. These increases were also strongly positively correlated with the ability to produce IL-4, IL-5, and IL-13 in vitro.

The present explanation related to the association between helminth infection and reduced allergic reactivity, based on studies in both mice and humans, is that parasite-driven production of IL-10, along with moderate increases in the frequency of Tregs, is responsible for the modulation of allergic reactivity (9–11). The ability of the Tregs to suppress immune-mediated pathology (38) was suggested in studies in mice infected with the intestinal nematode *Heligmosomoides polygyrus*, in which this parasite induced an expansion of natural Tregs (39–42) that prevented allergen-induced pathology (43, 44).

However, the present study suggests that on a background of allergic sensitization, immune responses to parasite Ags may actually be accentuated in patients with relatively acute filarial infections. This accentuated immune responsiveness may either lead to 1) control of the parasite and/or resistance to reinfection, or 2) alteration of the helminth infection–associated pathology.

The Th2-dominant immune response as observed in the  $\text{Fil}^+\text{A}^+$  in this study has been the object of discussion for many years in the context of both allergy and helminth infections. IL-4, the major cytokine that induces IgE isotype switching, has been implicated as being essential for the clearance/control of microfilariae in a murine infection model (45). Moreover, it has been postulated that helminth-induced alternatively activated macrophages that are driven by IL-4 and/or IL-13 have been shown to play an important role in the control of Th1-type inflammation, wound healing, and worm expulsion (46, 47).

IgE levels have also been associated with immunity to some helminth infections (48, 49). Indeed, Fitzsimmons et al. (50) reviewed a number of studies in human schistosomiasis, in which levels of parasite-specific IgE were shown to be correlated with resistance to reinfection (48, 51–55). Moreover, parasite-specific IgE responses have also been associated with immunity in human infections with hookworms (56, 57), *Trichuris* (58), and *Ascaris* infection (59, 60). Additionally, eosinophils and their granule proteins have been implicated in host defense against helminth parasites (61) and in mediating resistance to helminth infections, either alone or in conjunction with Ab, complement, or innate cells (62–66).

In contrast, this marked increase in the Th2-associated IgE levels and eosinophilia driven by filarial Ags in  $\text{Fil}^+\text{A}^+$  patients has also been implicated in the pathogenesis of some syndromes associated with filarial infections (67–70), including tropical pulmonary eosinophilia (68, 71, 72) and the localized hyperreactive dermatitis (sowda) seen in *Onchocerca volvulus*–infected patients (73–75). Finally, given that IL-10–mediated modulation of Th1/Th2/Th17/Th9 responses (76) has been associated with clinically asymptomatic filarial infection, it is possible that allergic sensitization coincident with these filarial infections results in altering the balance of regulatory and effector responses that could in turn alter the longevity of the parasites in their human hosts.

Taken together, our data suggest that allergic sensitization coincident with filarial infection drives a hyperreactive parasite Ag-specific T cell response characterized by an augmented Th2-associated immune response, eosinophilia, and elevated serum levels of IgE that might limit parasite burden but, as a consequence, allow for immune-mediated, parasite-associated morbidity.

## Disclosures

The authors have no financial conflicts of interest.

## References

- Feasey, N., M. Wansbrough-Jones, D. C. Mabey, and A. W. Solomon. 2010. Neglected tropical diseases. *Br. Med. Bull.* 93: 179–200.
- Pawankar, R., G. W. Canonica, S. T. Holgate, and R. F. Lockey. 2012. Allergic diseases and asthma: a major global health concern. *Curr. Opin. Allergy Clin. Immunol.* 12: 39–41.
- Pulendran, B., and D. Artis. 2012. New paradigms in type 2 immunity. *Science* 337: 431–435.
- Allen, J. E., and R. M. Maizels. 2011. Diversity and dialogue in immunity to helminths. *Nat. Rev. Immunol.* 11: 375–388.
- Cooper, P. J., M. E. Chico, L. C. Rodrigues, M. Ordonez, D. Strachan, G. E. Griffin, and T. B. Nutman. 2003. Reduced risk of atopy among school-age children infected with geohelminth parasites in a rural area of the tropics. *J. Allergy Clin. Immunol.* 111: 995–1000.
- Strachan, D. P. 1989. Hay fever, hygiene, and household size. *BMJ* 299: 1259–1260.
- van den Biggelaar, A. H., C. Lophuhaar, R. van Ree, J. S. van der Zee, J. Jans, A. Hoek, B. Migombet, S. Bormann, D. Luckner, P. G. Kremsner, and M. Yazdanbakhsh. 2001. The prevalence of parasite infestation and house dust mite sensitization in Gabonese schoolchildren. *Int. Arch. Allergy Immunol.* 126: 231–238.
- Cooper, P. J., M. E. Chico, M. Bland, G. E. Griffin, and T. B. Nutman. 2003. Allergic symptoms, atopy, and geohelminth infections in a rural area of Ecuador. *Am. J. Respir. Crit. Care Med.* 168: 313–317.
- Yazdanbakhsh, M., and S. Wahyuni. 2005. The role of helminth infections in protection from atopic disorders. *Curr. Opin. Allergy Clin. Immunol.* 5: 386–391.
- Mitre, E., D. Chien, and T. B. Nutman. 2008.  $\text{CD4}^+$  (and not  $\text{CD25}^+$ ) T cells are the predominant interleukin-10-producing cells in the circulation of filaria-infected patients. *J. Infect. Dis.* 197: 94–101.
- Metenou, S., B. Dembele, S. Konate, H. Dolo, S. Y. Coulibaly, Y. I. Coulibaly, A. A. Diallo, L. Soumaoro, M. E. Coulibaly, D. Sanogo, et al. 2010. At homeostasis filarial infections have expanded adaptive T regulatory but not classical Th2 cells. *J. Immunol.* 184: 5375–5382.
- Dold, S., J. Heinrich, H. E. Wichmann, and M. Wjst. 1998. Ascaris-specific IgE and allergic sensitization in a cohort of school children in the former East Germany. *J. Allergy Clin. Immunol.* 102: 414–420.
- Palmer, L. J., J. C. Celedón, S. T. Weiss, B. Wang, Z. Fang, and X. Xu. 2002. Ascaris lumbricoides infection is associated with increased risk of childhood asthma and atopy in rural China. *Am. J. Respir. Crit. Care Med.* 165: 1489–1493.
- Hunninghake, G. M., M. E. Soto-Quiros, L. Avila, N. P. Ly, C. Liang, J. S. Sylvia, B. J. Klanderman, E. K. Silverman, and J. C. Celedón. 2007. Sensitization to *Ascaris lumbricoides* and severity of childhood asthma in Costa Rica. *J. Allergy Clin. Immunol.* 119: 654–661.
- Steel, C., and T. B. Nutman. 2011. Altered T cell memory and effector cell development in chronic lymphatic filarial infection that is independent of persistent parasite antigen. *PLoS One* 6: e19197.
- Santiago, H. C., S. Bennuru, A. Boyd, M. Eberhard, and T. B. Nutman. 2011. Structural and immunologic cross-reactivity among filarial and mite tropomyosin: implications for the hygiene hypothesis. *J. Allergy Clin. Immunol.* 127: 479–486.
- Maizels, R. M., and M. Yazdanbakhsh. 2003. Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nat. Rev. Immunol.* 3: 733–744.
- Babu, S., and T. B. Nutman. 2014. Immunology of lymphatic filariasis. *Parasite Immunol.* 36: 338–346.
- Chatterjee, S., and T. B. Nutman. 2015. Helminth-induced immune regulation: implications for immune responses to tuberculosis. *PLoS Pathog.* 11: e1004582.
- Santiago, H. C., E. LeeVan, S. Bennuru, F. Ribeiro-Gomes, E. Mueller, M. Wilson, T. Wynn, D. Garboczi, J. Urban, E. Mitre, and T. B. Nutman. 2012. Molecular mimicry between cockroach and helminth glutathione S-transferases promotes cross-reactivity and cross-sensitization. *J. Allergy Clin. Immunol.* 130: 248–256.e9.
- Makiya, M. A., J. A. Herrick, P. Khoury, C. P. Prussin, T. B. Nutman, and A. D. Klion. 2014. Development of a suspension array assay in multiplex for the simultaneous measurement of serum levels of four eosinophil granule proteins. *J. Immunol. Methods* 411: 11–22.
- Liu, A. H., and D. Y. Leung. 2006. Renaissance of the hygiene hypothesis. *J. Allergy Clin. Immunol.* 117: 1063–1066.
- Hagel, I., M. Cabrera, M. A. Hurtado, P. Sanchez, F. Puccio, M. C. Di Prisco, and M. Palenque. 2007. Infection by *Ascaris lumbricoides* and bronchial hyper reactivity: an outstanding association in Venezuelan school children from endemic areas. *Acta Trop.* 103: 231–241.
- Wördemann, M., R. J. Diaz, L. M. Heredia, A. M. Collado Madurga, A. Ruiz Espinosa, R. C. Prado, I. A. Millan, A. Escobedo, L. Rojas Rivero, B. Gryseels, et al. 2008. Association of atopy, asthma, allergic rhinoconjunctivitis, atopic dermatitis and intestinal helminth infections in Cuban children. *Trop. Med. Int. Health* 13: 180–186.
- van den Biggelaar, A. H., R. van Ree, L. C. Rodrigues, B. Lell, A. M. Deelder, P. G. Kremsner, and M. Yazdanbakhsh. 2000. Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. *Lancet* 356: 1723–1727.
- van der Werff, S. D., J. W. Twisk, M. Wördemann, M. Campos Ponce, R. Diaz, F. A. Junco Núñez, L. Rojas Rivero, M. Bonet Gorbéa, and K. Polman. 2013. Deworming is not a risk factor for the development of atopic diseases: a longitudinal study in Cuban school children. *Clin. Exp. Allergy* 43: 665–671.
- Cooper, P. J., M. L. Barreto, and L. C. Rodrigues. 2006. Human allergy and geohelminth infections: a review of the literature and a proposed conceptual model to guide the investigation of possible causal associations. *Br. Med. Bull.* 79–80: 203–218.

28. Fallon, P. G., and N. E. Mangan. 2007. Suppression of Th2-type allergic reactions by helminth infection. *Nat. Rev. Immunol.* 7: 220–230.
29. Cooper, P. J. 2009. Interactions between helminth parasites and allergy. *Curr. Opin. Allergy Clin. Immunol.* 9: 29–37.
30. Teixeira-Carvalho, A., R. T. Fujiwara, E. J. Stemmy, D. Olive, J. M. Damsker, A. Loukas, R. Corrêa-Oliveira, S. L. Constant, and J. M. Bethony. 2008. Binding of excreted and/or secreted products of adult hookworms to human NK cells in *Necator americanus*-infected individuals from Brazil. *Infect. Immun.* 76: 5810–5816.
31. Fujiwara, R. T., G. G. Cançado, P. A. Freitas, H. C. Santiago, C. L. Massara, O. Dos Santos Carvalho, R. Corrêa-Oliveira, S. M. Geiger, and J. Bethony. 2009. *Necator americanus* infection: a possible cause of altered dendritic cell differentiation and eosinophil profile in chronically infected individuals. *PLoS Negl. Trop. Dis.* 3: e399.
32. Gazzinelli-Guimarães, P. H., E. M. Souza-Fagundes, G. G. Cancado, V. G. Martins, L. C. Dhom-Lemos, N. D. Ricci, J. A. Fiuza, L. L. Bueno, R. R. Miranda, S. Guatimosim, et al. 2013. Cell apoptosis induced by hookworm antigens: a strategy of immunomodulation. *Front. Biosci. (Elite Ed.)* 5: 662–675.
33. King, C. L., S. Mahanty, V. Kumaraswami, J. S. Abrams, J. Regunathan, K. Jayaraman, E. A. Ottesen, and T. B. Nutman. 1993. Cytokine control of parasite-specific anergy in human lymphatic filariasis. Preferential induction of a regulatory T helper type 2 lymphocyte subset. *J. Clin. Invest.* 92: 1667–1673.
34. Geiger, S. M., C. L. Massara, J. Bethony, P. T. Soboslay, and R. Corrêa-Oliveira. 2004. Cellular responses and cytokine production in post-treatment hookworm patients from an endemic area in Brazil. *Clin. Exp. Immunol.* 136: 334–340.
35. Ricci, N. D., J. A. Fiuza, L. L. Bueno, G. G. Cançado, P. H. Gazzinelli-Guimarães, V. G. Martins, L. F. Matoso, R. R. de Miranda, S. M. Geiger, R. Correa-Oliveira, et al. 2011. Induction of CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> regulatory T cells during human hookworm infection modulates antigen-mediated lymphocyte proliferation. *PLoS Negl. Trop. Dis.* 5: e1383.
36. Taylor, M. D., L. LeGoff, A. Harris, E. Malone, J. E. Allen, and R. M. Maizels. 2005. Removal of regulatory T cell activity reverses hyporesponsiveness and leads to filarial parasite clearance in vivo. *J. Immunol.* 174: 4924–4933.
37. Yazdanbakhsh, M., P. G. Kremsner, and R. van Ree. 2002. Allergy, parasites, and the hygiene hypothesis. *Science* 296: 490–494.
38. Maizels, R. M., A. Balic, N. Gomez-Escobar, M. Nair, M. D. Taylor, and J. E. Allen. 2004. Helminth parasites—masters of regulation. *Immunol. Rev.* 201: 89–116.
39. Finney, C. A., M. D. Taylor, M. S. Wilson, and R. M. Maizels. 2007. Expansion and activation of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells in *Heligmosomoides polygyrus* infection. *Eur. J. Immunol.* 37: 1874–1886.
40. Setiawan, T., A. Metwali, A. M. Blum, M. N. Ince, J. F. Urban, Jr., D. E. Elliott, and J. V. Weinstock. 2007. *Heligmosomoides polygyrus* promotes regulatory T-cell cytokine production in the murine normal distal intestine. *Infect. Immun.* 75: 4655–4663.
41. Rausch, S., J. Huehn, D. Kirchhoff, J. Rzepecka, C. Schnoeller, S. Pillai, C. Loddikenper, A. Scheffold, A. Hamann, R. Lucius, and S. Hartmann. 2008. Functional analysis of effector and regulatory T cells in a parasitic nematode infection. *Infect. Immun.* 76: 1908–1919.
42. Hang, L., A. M. Blum, T. Setiawan, J. P. Urban, Jr., K. M. Stoyanoff, and J. V. Weinstock. 2013. *Heligmosomoides polygyrus* bakeri infection activates colonic Foxp3<sup>+</sup> T cells enhancing their capacity to prevent colitis. *J. Immunol.* 191: 1927–1934.
43. Wilson, M. S., M. D. Taylor, A. Balic, C. A. Finney, J. R. Lamb, and R. M. Maizels. 2005. Suppression of allergic airway inflammation by helminth-induced regulatory T cells. *J. Exp. Med.* 202: 1199–1212.
44. Zaiss, D. M., J. van Loosdregt, A. Gorlani, C. P. Bekker, A. Gröne, M. Sibilia, P. M. van Bergen en Henegouwen, R. C. Roovers, P. J. Coffey, and A. J. Sijts. 2013. Amphiregulin enhances regulatory T cell-suppressive function via the epidermal growth factor receptor. *Immunity* 38: 275–284.
45. Volkman, L., M. Saeftel, O. Bain, K. Fischer, B. Fleischer, and A. Hoerauf. 2001. Interleukin-4 is essential for the control of microfilariae in murine infection with the filaria *Litomosoides sigmodontis*. *Infect. Immun.* 69: 2950–2956.
46. Kreider, T., R. M. Anthony, J. F. Urban, Jr., and W. C. Gause. 2007. Alternatively activated macrophages in helminth infections. *Curr. Opin. Immunol.* 19: 448–453.
47. Babu, S., V. Kumaraswami, and T. B. Nutman. 2009. Alternatively activated and immunoregulatory monocytes in human filarial infections. *J. Infect. Dis.* 199: 1827–1837.
48. Rihet, P., C. E. Demeure, A. Bourgois, A. Prata, and A. J. Dessein. 1991. Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. *Eur. J. Immunol.* 21: 2679–2686.
49. Dunne, D. W., A. E. Butterworth, A. J. Fulford, H. C. Kariuki, J. G. Langley, J. H. Ouma, A. Capron, R. J. Pierce, and R. F. Sturrock. 1992. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *Eur. J. Immunol.* 22: 1483–1494.
50. Fitzsimmons, C. M., F. H. Falcone, and D. W. Dunne. 2014. Helminth allergens, parasite-specific IgE, and its protective role in human immunity. *Front. Immunol.* 5: 61.
51. Hagan, P., U. J. Blumenthal, D. Dunn, A. J. Simpson, and H. A. Wilkins. 1991. Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. *Nature* 349: 243–245.
52. Dunne, D. W., A. E. Butterworth, A. J. Fulford, J. H. Ouma, and R. F. Sturrock. 1992. Human IgE responses to *Schistosoma mansoni* and resistance to reinfection. *Mem. Inst. Oswaldo Cruz* 87(Suppl. 4): 99–103.
53. Dunne, D. W., M. Webster, P. Smith, J. G. Langley, B. A. Richardson, A. J. Fulford, A. E. Butterworth, R. F. Sturrock, H. C. Kariuki, and J. H. Ouma. 1997. The isolation of a 22 kDa band after SDS-PAGE of *Schistosoma mansoni* adult worms and its use to demonstrate that IgE responses against the antigen(s) it contains are associated with human resistance to reinfection. *Parasite Immunol.* 19: 79–89.
54. Jiz, M., J. F. Friedman, T. Leenstra, B. Jarilla, A. Pablo, G. Langdon, S. Pond-Tor, H. W. Wu, D. Manalo, R. Olveda, et al. 2009. Immunoglobulin E (IgE) responses to paramyosin predict resistance to reinfection with *Schistosoma japonicum* and are attenuated by IgG4. *Infect. Immun.* 77: 2051–2058.
55. Fitzsimmons, C. M., F. M. Jones, A. Pinot de Moira, A. V. Protasio, J. Khalife, H. A. Dickinson, E. M. Tukaheba, and D. W. Dunne. 2012. Progressive cross-reactivity in IgE responses: an explanation for the slow development of human immunity to schistosomiasis? *Infect. Immun.* 80: 4264–4270.
56. Pritchard, D. I., R. J. Quinnell, and E. A. Walsh. 1995. Immunity in humans to *Necator americanus*: IgE, parasite weight and fecundity. *Parasite Immunol.* 17: 71–75.
57. Bethony, J., A. Loukas, M. Smout, S. Brooker, S. Mendez, J. Plieskatt, G. Goud, M. E. Bottazzi, B. Zhan, Y. Wang, et al. 2005. Antibodies against a secreted protein from hookworm larvae reduce the intensity of hookworm infection in humans and vaccinated laboratory animals. *FASEB J.* 19: 1743–1745.
58. Faulkner, H., J. Turner, J. Kamgno, S. D. Pion, M. Boussinesq, and J. E. Bradley. 2002. Age- and infection intensity-dependent cytokine and antibody production in human trichuriasis: the importance of IgE. *J. Infect. Dis.* 185: 665–672.
59. McSharry, C., Y. Xia, C. V. Holland, and M. W. Kennedy. 1999. Natural immunity to *Ascaris lumbricoides* associated with immunoglobulin E antibody to ABA-1 allergen and inflammation indicators in children. *Infect. Immun.* 67: 484–489.
60. Turner, J. D., H. Faulkner, J. Kamgno, M. W. Kennedy, J. Behnke, M. Boussinesq, and J. E. Bradley. 2005. Allergen-specific IgE and IgG4 are markers of resistance and susceptibility in a human intestinal nematode infection. *Microbes Infect.* 7: 990–996.
61. Specht, S., M. Saeftel, M. Arndt, E. Endl, B. Dubben, N. A. Lee, J. J. Lee, and A. Hoerauf. 2006. Lack of eosinophil peroxidase or major basic protein impairs defense against murine filarial infection. *Infect. Immun.* 74: 5236–5243.
62. Kazura, J. W., and M. Aikawa. 1980. Host defense mechanisms against *Trichinella spiralis* infection in the mouse: eosinophil-mediated destruction of newborn larvae in vitro. *J. Immunol.* 124: 355–361.
63. Lee, T. D. 1991. Helminthotoxic responses of intestinal eosinophils to *Trichinella spiralis* newborn larvae. *Infect. Immun.* 59: 4405–4411.
64. Rainbird, M. A., D. Macmillan, and E. N. Meeusen. 1998. Eosinophil-mediated killing of *Haemonchus contortus* larvae: effect of eosinophil activation and role of antibody, complement and interleukin-5. *Parasite Immunol.* 20: 93–103.
65. Venturiello, S. M., G. H. Giambartolomei, and S. N. Costantino. 1995. Immune cytotoxic activity of human eosinophils against *Trichinella spiralis* newborn larvae. *Parasite Immunol.* 17: 555–559.
66. Galioto, A. M., J. A. Hess, T. J. Nolan, G. A. Schad, J. J. Lee, and D. Abraham. 2006. Role of eosinophils and neutrophils in innate and adaptive protective immunity to larval strongyloides stercoralis in mice. *Infect. Immun.* 74: 5730–5738.
67. Ottesen, E. A., and T. B. Nutman. 1992. Tropical pulmonary eosinophilia. *Annu. Rev. Med.* 43: 417–424.
68. O'Bryan, L., P. Pinkston, V. Kumaraswami, V. Vijayan, G. Yenokida, H. F. Rosenberg, R. Crystal, E. A. Ottesen, and T. B. Nutman. 2003. Localized eosinophil degranulation mediates disease in tropical pulmonary eosinophilia. *Infect. Immun.* 71: 1337–1342.
69. Nutman, T. B. 2007. Evaluation and differential diagnosis of marked, persistent eosinophilia. *Immunol. Allergy Clin. North Am.* 27: 529–549.
70. Lobos, E., T. B. Nutman, J. S. Hothersall, and S. Moncada. 2003. Elevated immunoglobulin E against recombinant *Brugia malayi*  $\gamma$ -glutamyl transpeptidase in patients with bancroftian filariasis: association with tropical pulmonary eosinophilia or putative immunity. *Infect. Immun.* 71: 747–753.
71. Nutman, T. B., V. K. Vijayan, P. Pinkston, V. Kumaraswami, C. Steel, R. G. Crystal, and E. A. Ottesen. 1989. Tropical pulmonary eosinophilia: analysis of antifilarial antibody localized to the lung. *J. Infect. Dis.* 160: 1042–1050.
72. Marshall, B. G., R. J. Wilkinson, and R. N. Davidson. 1998. Pathogenesis of tropical pulmonary eosinophilia: parasitic alveolitis and parallels with asthma. *Respir. Med.* 92: 1–3.
73. Rubio de Krömer, M. T., C. E. Medina-De la Garza, and N. W. Brattig. 1995. Differences in eosinophil and neutrophil chemotactic responses in sowda and generalized form of onchocerciasis. *Acta Trop.* 60: 21–33.
74. Hoerauf, A., S. Kruse, N. W. Brattig, A. Heinzmann, B. Mueller-Myhsok, and K. A. Deichmann. 2002. The variant Arg110Gln of human IL-13 is associated with an immunologically hyper-reactive form of onchocerciasis (sowda). *Microbes Infect.* 4: 37–42.
75. Katawa, G., L. E. Layland, A. Y. Debrah, C. von Horn, L. Batsa, A. Kwarteng, S. Ariens, D. W. Taylor, S. Specht, A. Hoerauf, and T. Adjibimey. 2015. Hyperreactive onchocerciasis is characterized by a combination of Th17-Th2 immune responses and reduced regulatory T cells. *PLoS Negl. Trop. Dis.* 9: e3414.
76. Babu, S., C. P. Blauvelt, V. Kumaraswami, and T. B. Nutman. 2006. Regulatory networks induced by live parasites impair both Th1 and Th2 pathways in patent lymphatic filariasis: implications for parasite persistence. *J. Immunol.* 176: 3248–3256.