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Filarial Infection Modulates the Immune Response to Mycobacterium tuberculosis through Expansion of CD4+ IL-4 Memory T Cells

Soumya Chatterjee,* Carolyn E. Clark,* Enrico Lugli,†,1 Mario Roederer,† and Thomas B. Nutman*‡

Exaggerated CD4+ T helper 2–specific cytokine producing memory T cell responses developing concomitantly with a T helper 1 response may have a detrimental role in immunity to infection caused by Mycobacterium tuberculosis. To assess the dynamics of Ag-specific memory T cell compartments in the context of filarial infection, we used multiparameter flow cytometry on PBMCs from 25 microfilariean filarial-infected (Inf) and 14 filarial-uninfected (Uninf) subjects following stimulation with filarial Ag (BmA) or with the M. tuberculosis–specific Ag culture filtrate protein-10 (CFP-10). Our data demonstrated that the Inf group had a marked increase in BmA-specific CD4+IL-4+ cells (median net frequency compared with baseline [Fo] = 0.09% versus 0.01%; \( p = 0.038 \)) but also to CFP-10 (Fo = 0.16% versus 0.007%; \( p = 0.04 \)) and staphylococcal enterotoxin B (Fo = 0.49% versus 0.26%; \( p = 0.04 \)). The Inf subjects showed a BmA-specific expansion of CD4+CD45RO+IL-4+ producing central memory (T<sub>CM</sub>, CD45RO+CCR7+CD27+; Fo = 1.1% versus 0.5%; \( p = 0.04 \)) as well as effector memory (T<sub>EM</sub>, CD45RO+CCR7−CD27−; Fo = 1.5% versus 0.2%; \( p = 0.03 \)) with a similar but nonsignificant response to CFP-10. In addition, there was expansion of CD4+IL-4+CD45RA−CCR7+CD27+ (naive-like) in Inf individuals compared with Uninf subjects. Among Inf subjects with definitive latent tuberculosis, there were no differences in frequencies of IL-4–producing cells within any of the memory compartments compared with the Uninf group. Our data suggest that filarial infection induces Ag-specific, exaggerated IL-4 responses in distinct T cell memory compartments to M. tuberculosis–specific Ags, which are attenuated in subjects who are able to mount a delayed type hypersensitivity reaction to M. tuberculosis. *The Journal of Immunology, 2015, 194: 000–000.

Filarial parasites infect an estimated 200 million people worldwide and have complex life cycles with the capacity for chronic persistence in the lymphatics or s.c. tissues of humans. Patent filarial infections (i.e., establishment of the adult worms and subsequent microfilariae [MF] production) are able to induce profound parasite Ag-specific CD4+ T cell hyporesponsiveness that is felt to be IL-10 dependent but also may reflect expansion of the Th2-like CD4+ T cells (IL-4, IL-5) (1, 2).

This imbalanced parasite Ag-driven CD4+ T cell response seen in chronic filarial infections can impose modulation of bystander Ag responses as well (3, 4) and becomes particularly relevant in endemic areas where there is significant geographic overlap with tuberculosis (TB), an infection that is a significant cause of morbidity and mortality worldwide (5). The immune modulation caused by these filarial parasites is of particular relevance in the current global search for an effective TB vaccine, especially after it has been demonstrated that bacillus Calmette–Guérin (BCG) is poorly protective for active pulmonary TB in parasite-endemic areas (6). Impaired cellular responses and increased Ag-driven IL-4 production to purified protein derivative (PPD) have been demonstrated previously in filarial infections (7, 8), but it is unclear how the parasite-induced expansion of Th2 cell populations might affect CD4-specific memory responses to concurrent infections such as TB.

It is also not well understood how these responses might be modulated in subjects who have the capability of generating a robust IFN-γ–specific T cell recall response to M. tuberculosis–specific Ags because of prior exposure to the mycobacteria, a response that currently defines the clinical state of latent TB infection (LTBI) (9). Models of chronic viral infection have provided some insights into the generation and persistence of various memory T cell populations (10, 11) and some phenotypic characterization of memory populations in humans with chronic filarial infections has been done previously (12).

There is, however, a paucity of studies looking at functional Ag–specific immune responses of these memory populations in the setting of chronic helminth infections. Using recent advances in multiparameter flow cytometry, we sought to combine phenotyping with Ag-specific cytokine production. We hypothesized that the filarial infection-induced expansion of specific CD4+ subtypes modulate the quality and nature of the CD4+ T cell responses to M. tuberculosis. To this end, we sought to examine specific

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Abbreviations used in this article: BCG, bacillus Calmette–Guérin; BmA, filarial Ag; CFP-10, M. tuberculosis–specific Ag culture filtrate protein-10; Fo, median frequency compared with baseline; IGRA, IFN-γ release assay; Inf, filarial-infected; LTBI, latent TB infection; MF, microfilariae; NV, naive-like; PPD, purified protein derivative; SEB, Staphylococcus aureus enterotoxin B; TB, tuberculosis; T<sub>EM</sub>, effector memory cell; TEMRA, RA+ T effector memory cell; Uninf, filarial-uninfected.
memory CD4+ T cell responses to both filarial Ag and to M. tuberculosis–specific Ag culture filtrate protein-10 (CFP-10) in filarial-infected (Inf) individuals and in filarial-uninfected (Uninf) controls. In addition, we compared the effect of having developed a delayed-type hypersensitivity response to CFP-10 on these responses by comparing subjects with or without LTBI in the Inf group. To our knowledge, our data provide the first detailed characterization of filarial infection induced Ag-specific, exaggerated IL-4 responses in specific T cell memory compartments to M. tuberculosis–specific Ags. We show, specifically, that this expansion predominantly affects early precursor cells with a naive-like phenotype and that responses within this compartment are attenuated in subjects who are able to mount a delayed type hypersensitivity reaction to M. tuberculosis.

Materials and Methods

Study population

Twenty-five microfilaremic patients with Loa loa (Inf) were studied, as were 14 filaria-uninfected healthy controls (Uninf) with no evidence of prior exposure to filarial infection (Table I). Inf individuals were from different countries in western and central Africa (Table I), whereas the healthy volunteers (Uninf) were U.S. residents with no history of travel or exposure to filarial infections. All individuals were examined and samples collected as part of registered protocols approved by the Institutional Review Boards of National Institute of Allergy and Infectious Diseases (NCT00001230 and NCT00001345) for the filarial infected patients and of Department of Transfusion Medicine, Clinical Center, National Institutes of Health (NCT0099-C0168) for the healthy donors. Written informed consent was obtained from all subjects.

Microfilaremia was detected in 1 ml of anticoagulated blood following previously established protocols (13). Quantiferon-TB Gold In-Tube (Cellelis, Valencia, CA; IFN-γ release assay [IGRA]) was used to diagnose LTBI. Filarial Ag (BmA)-specific IgG4 and IgG ELISA were performed exactly as previously described (14). All filarial-infected donors had normal chest radiographs with no pulmonary symptoms (fever, cough, chest pain, or hemoptysis).

Ags and In Vitro Culture

BmA, obtained from saline extracts of Brugia malayi adult worms, and CFP-10 (Fitzgerald Industries International, Acton, MA) were used as the antigenic stimuli. Final concentrations were 10 μg/ml for CFP-10. Staphylococcus enterotoxin B (SEB; Toxin Technology, Sarasota, FL) at a final concentration of 1 μg/ml was used as positive control. Cultures on PBMCs were performed to determine memory subsets and levels of intracellular cytokines. Briefly, cells were plated in RPMI 1640, with 10% FCS with penicillin–streptomycin (100 U per 100 mg/ml), L-glutamine (2 mM) media at a maximum of 2 × 10^6/well, in a volume of 200 μl/well, in a 96-well round bottom plate with BmA, CFP-10 or SEB as well as media alone in the presence of α-CD28/CD49d beads (Invitrogen) at a final concentration of 1 μg/ml, used as costimulatory molecules. FastImmune Brefeldin A solution (1 μg/ml) was added after 2 h of culture. Cells were cultured overnight (16–18 h) and were then harvested, washed, and stained for flow cytometry.

Flow cytometry and intracellular cytokine staining

Flow cytometry acquisition was performed on a modified LSRII instrument (BD biosciences) with 18 fluorescent parameter detection capabilities. Compensation and analysis of data were performed using Flowjo software (Tree Star). Unlabeled Abs were conjugated at the Immunotechnology Section, Vaccine Research Center, NIAID, as previously reported (http://www.dmr.com/about/). BmA-specific IgG (median, 4978 ng/ml; range, 782–65,950 ng/ml). In contrast the controls had no measurable BmA-specific IgG. None had any pulmonary symptoms or fever at the time of assessment and all but two subjects (with calcified nodules <7 mm) had normal chest radiographic findings (data not shown). IGRA were positive in six infected subjects, and none of the controls was positive. All subjects in the Inf and Uninf groups were HIV negative. No differences were seen in frequencies of different CD4+ memory compartments (TCM, TEM, NV, TEMRA) between Inf and Uninf subjects (Table II). Therefore, age, sex, and WBC count distributions between Inf and Uninf subjects were noted to be broadly similar, but Inf subjects were all microfilaremic (median count 180) and all had serologic evidence of filarial infection, with 72% (18/25) having evidence of eosinophilia.

Filarial infection is associated with increased frequencies of IL-4-producing CD4+ T cells in response to BmA, CFP-10 and SEB

To delineate steady state and Ag-driven CD4+ T cell cytokine–producing cells (IFN-γ, TNF-α, IL-4 and IL-10), we cultured PBMCs with media alone and in response to stimulation by BmA, CFP-10 and superantigen SEB. Although at baseline no differences were noted between Inf and Uninf in the frequencies of CD4+ T cells producing IFN-γ,TNF-α, IL-4 or IL-10 (Fig. 1), in the Inf group, increased Ag induced frequencies of IL-4–producing CD4+ T cells (expressed as net frequency compared with baseline as defined above) were noted in response to BmA compared with the Uninf group (median net frequency compared with baseline [Fo] = 0.09% versus 0.01%; p = 0.038). Similar differences were seen in CD4+IL-4+ frequencies in response to CFP-10 (Fo = 0.16% versus 0.007%; p = 0.04) and to SEB (Fo = 0.49% versus 0.26%; p = 0.04). No differences were noted in the Fo of CD4+IFN-γ–producing cells between Inf and Uninf groups in response to BmA, CFP-10, or SEB. Although at baseline a nonsignificant increase in the Fo of CD4+ TNF-α–producing cells was in the Inf

Results

Study populations

The Inf patients were all microfilariae positive with MF counts that ranged from 2 to 10,400 MF/ml (median, 180 MF/ml) whereas the Uninf patients were MF negative. As outlined in Table I, the Inf group had a gender distribution of 72% male and 28% female, with an age range of 24–62 years (median, 34 years). The filarial-uninfected (Uninf) group came from a subset of donors whose gender distribution was 57% male and 43% female with an age range of 25–81 years (median, 45 years). There was no difference in age and gender distribution between the two groups. For the Inf group, there was no elevation in total WBC counts (median, 7.08) or lymphocyte counts (not shown) but 18/25 had absolute eosinophil counts > 500/ml (range 410-1224). Inf subjects had elevated BmA-specific IgG4 (median, 4978 ng/ml; range, 782–65,950 ng/ml). Therefore, age, sex, and WBC count distributions between Inf and Uninf subjects were noted to be broadly similar, but Inf subjects were all microfilaremic (median count 180) and all had serologic evidence of filarial infection, with 72% (18/25) having evidence of eosinophilia.

Statistical analysis

Data analyses were performed using GraphPad PRISM (GraphPad Software, San Diego, CA, USA). Median frequencies were used for measurements of central tendency. Statistically significant differences between two groups were analyzed using the nonparametric Mann–Whitney U test and the Kruskal–Wallis test with Dunn multiple comparison test was used for multiple comparisons. p
Specific CD4+IL-4+ responses were noted in the Inf group compared to the Uninf group at baseline, with increased parasite and TEM, NV, and TEMRA compartments in the Inf group versus the Uninf group. These differences were also seen with SEB.

Ag-specific IL-4 is induced in specific CD4+ T cell memory compartments

To define further the nature of the increased Ag-driven IL-4 responses in CD4+ memory compartment, we compared the frequencies of CD4+IL-4+ cells between infected subjects and uninfected controls in four different memory compartments (TCM, TEM, NV, and TEMRA). As shown in Fig. 2 there was marked expansion of IL-4+CD4+ T cells in the central memory (TCM) compartment in the Inf group compared with Uninf (Fo = 0.09% versus 0.03%; p = 0.029) and effector memory (TEM) compartment (Fo = 0.1% in Inf versus 0.02% in Uninf; p = 0.017) in response to BmA. Interestingly, a similar trend toward increased IL-4 production was also noted in cells with an NV phenotype (Fo = 0.03% in Inf versus 0.01% in Uninf; p = 0.12). Significantly increased IL-4–producing CD4+ cell frequencies were noted in the NV compartment in response to the M. tuberculosis–specific Ag CFP-10 (Fo = 0.06% in Inf versus 0.002% in Uninf; p = 0.027) with similar but nonsignificant increases in frequencies of TCM and TEM IL-4–producing cells. In contrast, the increased IL-4 response to SEB was primarily limited to the TEM compartment (Fo = 0.96% in Inf versus 0.51% in Uninf; p = 0.03). In the Inf group, therefore, parasite and M. tuberculosis Ag-specific increase in CD4+IL-4+ frequencies was localized not only to TCM and TEM compartments, but also within cells of an NV phenotype, a pattern not seen on nonspecific T cell stimulation with SEB.

The pattern of preferential IL-4 expansion in the CD4+ memory compartment compared with Th1 cytokines (IFN-γ and TNF-α) showed similar trends in response to BmA and CFP-10 but not to SEB

To delineate the Th1/Th2 cytokine milieu within each memory CD4+ T cell compartment, we performed a comparative assessment of the Fo in Ag-specific frequencies of IL-4 with Th1-like cytokine (IFN-γ and TNF-α) producing CD4+ T cells in each individual memory compartments (Fig. 3) between Inf and Uninf groups after adjusting for baseline expression. Within the TCM and TEM pool, in response to BmA an expansion primarily of IL-4–producing cells was noted primarily in the Inf group. The Fo in Inf compared with Uninf subjects in TCM was 0.09% versus 0.03% (IL-4), 0.00085% versus 0.004% (IFN-γ), and 0.001% versus 0.001% (TNF-α). Fo in TEM was 0.1% versus 0.02% (IL-4), 0.004% versus 0.001% (IFN-γ), and 0.001% versus 0.02% (TNF-α). Similar results were seen in response to CFP-10 where the Fo in TCM in Inf versus Uninf was 0.16% versus 0.03% (IL-4), 0.002% versus 0.004% (IFN-γ) and 0.001 versus 0.01% (TNF-α). In TEM Fo in Inf versus Uninf was 0.03% versus 0.02% (IL-4), 0.01% versus 0.003% (IFN-γ) and 0.009% versus 0.02% (TNF-α). In the CD4+ NV compartment, a primarily IL-4–dominant response to BmA was noted in both Inf and Uninf groups, with the Fo being 1.04% versus 0.26% (IL-4), 0.06% versus 0.001% (IFN-γ), and 0.07 versus 0.001% (TNF-α). CFP-10–specific responses showed a similar IL-4 dominance in the NV compartment in the Inf group with the Fo of 0.06% (IL-4), 0.001% (IFN-γ), and 0.0001% (TNF-α). Increased Fo of TNF-α–producing cells were noted in the NV compartment in the Uninf group, with Fo of 0.002% (IL-4), 0.001% (IFN-γ), and 0.007% (TNF-α). Moreover, in response to SEB, expansion of IFN-γ and TNF-α but not IL-4–producing cells was noted in both Inf and Uninf groups in TCM Fo of 0.37% versus 0.43% for IL-4, 1.65% versus 1.643% for IFN-γ, and 2.72% versus 3.57% for TNF-α. A similar result

Table I. Baseline characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Inf</th>
<th>Uninf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (n)</td>
<td>25</td>
<td>14</td>
</tr>
<tr>
<td>Median age, y (range)</td>
<td>34 (24–62)</td>
<td>45 (25–81)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>18/7</td>
<td>8/6</td>
</tr>
<tr>
<td>Median duration of infection, y (range)</td>
<td>5 (1–18)</td>
<td>—</td>
</tr>
<tr>
<td>Country infection acquired</td>
<td>Nigeria (5)</td>
<td>—</td>
</tr>
<tr>
<td>Central African Republic (1)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Democratic Republic of Congo (4)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Quantiferon-TB Gold test (positive/negative/indeterminate)</td>
<td>69/10</td>
<td>0/9/5</td>
</tr>
<tr>
<td>Median total WBC count (range)</td>
<td>7.08 (4.08–18.4)</td>
<td>NA</td>
</tr>
<tr>
<td>Median absolute eosinophil count (range)</td>
<td>786 (410–1224)</td>
<td>NA</td>
</tr>
<tr>
<td>Positive IgE (units)</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Median microfilaria count (range)</td>
<td>180 (2–10,400)</td>
<td>0</td>
</tr>
<tr>
<td>BmA-specific IgG4 (ng/ml)</td>
<td>4978 (782–6,5950)</td>
<td>0</td>
</tr>
</tbody>
</table>

Table II. CD4+ immune profile showing memory subsets in Inf and Uninf groups expressed as percent CD4

<table>
<thead>
<tr>
<th>CD4+ T Cell Memory Profile (% CD4+ T Cells)</th>
<th>Inf Median (Range)</th>
<th>Uninf Median (Range)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive-like cells</td>
<td>48.05 (12.10–72)</td>
<td>37.10 (15.64–30)</td>
<td>0.14</td>
</tr>
<tr>
<td>RA+ T effectors</td>
<td>4.96 (2.07–17.70)</td>
<td>5.72 (1.69–20.6)</td>
<td>0.72</td>
</tr>
<tr>
<td>Central memory</td>
<td>28.65 (15.4–41.0)</td>
<td>29.8 (13.4–49.5)</td>
<td>0.45</td>
</tr>
<tr>
<td>Effector memory</td>
<td>15.10 (3.49–44.3)</td>
<td>18.4 (7.42–32.9)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

\*Naive-like cells (CD45RA⁺CCR7⁺CD27⁺), RA⁺ T effectors (CD45RA⁺CCR7⁻CD27⁻), central memory (CD45RO⁺CCR7⁻CD27⁻), and effector memory (CD45RO⁺CCR7⁺CD27⁻).
was seen in the NV compartment: Fo of 0.1% versus 0.06% (IL-4), 0.19% versus 0.12% (IFN-γ), and 1.09% versus 2.18% (TNF-α).

The increased IL-4 response to SEB in the Inf group versus the Uninf group was seen only in cells with a TEM phenotype (Fo = 0.96% versus 0.51% for IL-4, 1.6% versus 1.47% for IFN-γ, and 2.9% versus 2.6% for TNF-α. Therefore, when comparing the balance of Th1/Th2 responses to BmA and CFP-10, in the Inf group, Ag-specific IL-4 expansion occurred in the TEM pool, precursor memory populations (TCM), and the NV compartment, leading to diminished frequencies of Th1 cytokine-producing cells, whereas the Th1 response was largely intact in these compartments for the Uninf. The expansion of Th1 cytokines in response to T cell superantigen (SEB) in both groups demonstrated that there was no impairment of Th1 cytokine-producing capacity in both groups.

**IGRA positivity among the Inf group abrogated the increased IL-4 response to mycobacterial Ag**

To test whether LTBI status (defined on the basis-positive IGRA) in the Inf group makes any difference to the IL-4 response, we compared the net increase in IL-4-producing cell Fo in response to CFP-10 (Fig. 4) between those Inf subjects with LTBI (Q+Inf, n = 6) and the Uninf (Q-Uninf, n = 9) controls (who were IGRA negative). A similar comparison was performed for Inf subjects without LTBI (Q-Inf, n = 7) and the Q-Uninf group. The increased IL-4 response in the NV compartment was maintained only in the Q-Uninf (Fo = 0.0153%) group when compared with the Q-Uninf controls (Fo = 0.002%, p = 0.003). A similar IL-4 expansion was observed within the TCM compartment where the net frequency was 0.26% (Q-Inf) versus 0.03% (Q-Uninf; p = 0.03).

The increased IL-4 response was not seen when Q+Inf was compared with the Q-Uninf group for cells in the NV pool (Fo = 0.01% versus 0.002%, respectively) or in the TCM pool (Fo = 0.16% versus 0.034%, respectively). The CD4+IL-4+ Fo in parasite-infected subjects with indeterminate IGRA results (QInd, n = 10), although not statistically significant, was increased in the NV compartment (Fo = 0.05%) compared with the Q-Uninf (Fo = 0.002%) but was not different in the TCM compartment (QInd Fo = 0.029% versus Q-Uninf Fo = 0.03%). These data provide additional evidence that the Ag-specific IL-4 response in infected subjects was primarily derived from the NV compartment. No differences (data not shown) were noted between the QInd and Q-Uninf groups in CD4+IL-4+ cells within TEMRA or TEM compartments. We conclude, therefore, that parasite-infected subjects with the capacity to generate an optimal Th1 recall response by demonstrating IGRA positivity showed concomitantly attenuated Th2 responses, as demonstrated by the decreased frequencies of CD4+IL-4+ cells in the TCM and in the NV compartment. Inf subjects who were IGRA negative or had indeterminate results on IGRA testing continued to have increased frequencies of CD4+IL-4+ cells in the NV compartment, providing additional evidence that filarial infection might produce fundamental alterations in precursors of memory populations.
Discussion

One of the hallmarks of tissue invasive helminth infections in both humans and in animal models is the induction of an IL-4-domin-ated T cell response that occurs at the time the infection becomes patent (when fertilized adult female worms produce offspring, such as eggs in schistosomes and microfilariae in filarial infections). The present study clearly demonstrates that the expansion of IL-4-producing CD4+ T cells occurs in specific niches (central and effector memory) within the CD4+ memory T cell pool and that this IL-4 expansion affects CD4+ T cell memory responses to the M. tuberculosis–specific Ag CFP-10, and effector memory (TEM). Box and whisker plots represent median with 95% confidence intervals and individual dots representing each subject. Increase in CD4+ T cells producing IL-4 is expressed as net frequency (defined as Ag-induced frequency − baseline frequency) in the Inf (n = 25) and Uninf (n = 14) groups are shown in response to BmA, CFP-10, and SEB. Horizontal bars represent the median frequencies. The p values were calculated using the Mann–Whitney U test.

Our study showed that adult subjects with L. loa infection had an increased frequency of CD4+IL-4+ cells in response to BmA and to CFP-10. Chronic patent filarial infections are associated with impaired CD4+ T cell proliferative responses, and it is well known that these infections, similar to other helminth infections (16, 17) induce an expansion of the Th2 response with down-modulation of Th1-like responses (8, 18, 19). It has also been demonstrated that immunomodulation caused by helminths can led to exaggerated IL-4 responses to mycobacterial Ags like PPD in endemic subjects infected with Onchocerca volvulus (8) and in a murine model in which preimmunization with live MF of B. malayi or with BmA skewed the PPD-specific response such that increased IL-4 and IL-5 were produced in addition to IFN-γ (20). Similar results were obtained with L. loa infection, but the intensity of the IL-4 response was life cycle stage specific with increased responses noted to adult and MF stages compared with L3 Ag (21). In an adult B. malayi–infected population, however, no increase in detectable IL-4 in response to PPD was seen, which might reflect population differences in immune modulation by these parasites (22).

The role of an exaggerated IL-4 response in tuberculosis is still being elucidated. Murine models have failed to provide a consen-sus, with exaggerated Th2 responses leading to pathology being demonstrated in the BALB/c model in some studies (23), whereas others have shown no difference in the control of M. tuberculosis.
infection in IL-4+/− and STAT6+/− mice (24). In human studies, increased IL-4 gene expression in PBMCs has been shown to correlate with severity of symptoms, extent of radiographic disease, and number of cavities (25). In addition, increased IL-4 levels detected in bronchoalveolar lavage fluid correlate positively with sputum smear positivity (26). However, whether background Th2 expansion in humans as might happen in chronic filarial infections leads to increased susceptibility to M. tuberculosis will need large prospective studies that are challenging to perform. Nevertheless, it is tempting to speculate that an optimal vaccine against M. tuberculosis will not only have to induce an effective Th1 response as has been suggested from animal model studies (27, 28); it must also be able to modulate a Th2 response. It was interesting to note that the six subjects in the Uninf group with exaggerated IL-4 responses were all IGRA negative and were among those who had minimal Th1 type responses with low frequencies of CD4+ IFN-γ and CD4+TNF-α positive cells. Heightened IL-4 production might possibly have been the default response of these subjects who had no evidence of prior exposure to TB.

Recent advances in flow cytometry have allowed for phenotypic characterization of memory T cells using a well-defined set of markers. The CD45 (CD45RA and CD45RO) isoforms have been used successfully since the 1980s to distinguish naive human T cells from memory cells, with CD45RO being preferentially expressed on memory cells (29, 30). In addition, we further delineated subpopulations within each group using CD27, a member of the TNF receptor superfamily, and C-C chemokine receptor 7 (CCR7) to define memory and effector populations using a standardized set of criteria (31). Although the role of memory CD4+ T cells in long-term protection against chronic infections is not clear, long-lived CD4+ memory responses have been demonstrated in chronic human viral infections (32, 33). We did not see any differences in the ex vivo frequencies of individual memory populations, as has been demonstrated previously in subjects with chronic W. bancrofti infection (12). Combining phenotype with functionality, however, as was done in our study might be more relevant for infections like TB that depend directly on the effector function of CD4+ cells, because this might have implications for vaccination (34).

A limitation of our study, however, is the selective sampling of the peripheral blood compartment, similar to other studies on human memory cells. This method might underestimate tissue-specific immune responses that are probably more predictive of outcome in infections such as TB. Given the heterogeneity of memory CD4+ T cell populations, the specific memory subsets that might play a role in protection against TB are still being defined. In this regard it has been demonstrated that a TEM population dominates in subjects previously treated with antituberculous chemotherapy (35). In addition, a higher frequency of the CD45RA+CD27+ effector phenotype with high PD-1 expression was seen in subjects with latent TB.

**FIGURE 3.** Filaria-infected subjects show preferential IL-4 expansion (compared with IFN-γ and TNF-α) in the T_CM and T_EM compartments in response to BmA and CFP-10. In the NV compartment, Inf subjects have a dominant IL-4 response compared with Uninf subjects, who have a dominant TNF-α response. Data are represented as pie charts comparing median net frequency in CD4+ cytokine producing (IL-4, IFN-γ, and TNF-α) cells as parts of the whole response in the different memory compartments (NV, TEMRA, T_CM, T_EM) between Inf (n = 25) and Uninf (n = 14) groups in response to BmA, CFP-10, and SEB.
when compared with BCG-vaccinated individuals in whom a CD27+ early-stage population was dominant (36). CD4+ cells with an NV phenotype (CD44loCD62Lhi) have been described to confer protection to low-dose aerosol challenge on adoptive transfer to Rag2−/− mice (37). Whether these cells have the phenotype of the recently described memory cells with stem cell–like properties (38) remains to be determined. Selective M. tuberculosis Ag-specific Th2 expansion instead of protective Th1 responses within cells with an NV phenotype as seen in the parasite infected subjects might therefore have important implications in developing protective immunity to TB and optimal M. tuberculosis–specific vaccine response.

The generation and maintenance of an IL-4–specific Th2 memory pool and its specific role in different infectious states remains even less well understood. It has been demonstrated in vitro that the Th2 phenotype, once generated, can persist stably and irreversibly by repression of IL-12 receptor signaling (39) and that a short duration of Th2 development can generate memory populations with high-level Th2 cytokine production capabilities on recall activation (40). Persistence of Th2 memory with increased IL-4 responses on reinfection has been well demonstrated using IL-4 reporter mice infected with the gastrointestinal nematodes Heligmosomoides polygyrus (41) and Trichuris muris (42) even after clearance of parasite. Our study clearly demonstrates an increased Ag-driven IL-4–specific responses in the TCM and the TEM compartment to parasite Ag with a similar trend when stimulated with the CFP-10.

Interestingly, an increased IL-4 response was also seen in cells with an NV phenotype in response to CFP-10. This difference was not seen on polyclonal activation with SEB. This might possibly suggest an Ag-experienced, early-stage, IL-4–producing cell within the NV compartment. Alternatively, this might suggest a change in the transcriptional program within early-stage precursor cells in a STAT6-independent manner because of consistently high levels of IL-4 that are produced by different cell types in chronic helminth infections (43, 44). The mechanisms by which these cells upregulate IL-4 production on Ag stimulation remains ill-defined, as it appears that T-bet suppression rather than GATA-3 overexpression might be the mechanism of increased production of Th2-like cytokines in chronic filarial infections (45).

Finally, we noted that the increased IL-4 production in response to CFP-10 in the NV and TCM compartments was attenuated in the Inf subjects who were QuantiFERON-TB Gold positive. Presumably, such patients with LTBI have CD4+IFN-γ–producing memory populations recognizing immunodominant M. tuberculosis–specific Ags, possibly because of a state of periodic Th1-like antigenic stimulation (46). These results are consistent with the emerging hypothesis of CD4+ T cell plasticity where a proinflammatory environment and ongoing Ag-specific stimulation can alter the cytokine-producing potential of Th2 cells, a concept that has been elegantly shown in a LCMV infection model (47). This plasticity may be related in part to histone modifications of specific T-bet gene loci in non-Th1 cells that, under appropriate conditions, can be permissive or suppressive and enable these cells to switch phenotype (48).

By combining phenotypic characterization with functional cytokine responses, our study demonstrates how chronic persistent filarial infection might modulate compartment-specific memory

![FIGURE 4. LTBI status (defined as a positive QuantiFERON-TB Gold test) in Inf subjects abrogates the increased IL-4 response to CFP-10 in the NV and TCM compartments. Representative data are expressed as percent CD4+ cells expressing cytokines in the four different memory compartments: naive-like (NV), RA+ effector T cells (TEMRA), central memory (TCM), and effector memory (TEM). Box and whisker plots represent median with 95% confidence intervals, with individual dots representing each subject. The increase in CD4+ T cells producing IL-4 is expressed as net frequency (defined as Ag-induced frequency–baseline frequency). Responses to CFP-10 in healthy controls (Q-Uninf, n = 9) who were QuantiFERON-TB Gold test negative (n = 13) were compared separately with Inf subjects with positive (Q+Inf, n = 6) and negative (Q-Inf, n = 7) QuantiFERON-TB Gold test. Horizontal bars represent the median frequencies. The p values were calculated using the Mann-Whitney test.](http://www.jimmunol.org/ Downloaded from)
responses to parasite and M. tuberculosis—specific Ags in filarial-infected individuals. These findings may have significant implications in rational vaccine design for TB in endemic areas of the world where there is significant geographic overlap with helminth infections. The findings could also open the way to a broader understanding of the memory response in latent TB. Further understanding of Th1- and Th2-specific CD4+ memory populations in chronic infections and their regulatory pathways for generation and maintenance should lead to better strategies to control these infections.

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Disclosures

The authors have no financial conflicts of interest.

References


