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IL-10 and Its Related Superfamily Members IL-19 and IL-24 Provide Parallel/Redundant Immune-Modulation in *Loa loa* Infection

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Background. Interleukin-10 (IL-10) has been implicated as the major cytokine responsible for the modulation of parasite-specific responses in filarial infections; however, the role of other IL-10 superfamily members in filarial infection is less well studied.

Methods. Peripheral blood mononuclear cells from loiasis patients were stimulated with or without filarial antigen. Cytokine production was quantified using a Luminex platform and T-cell expression patterns were assessed by flow cytometry.

Results. All patients produced significant levels of IL-10, IL-13, IL-5, IL-4, and IL-9 in response to filarial antigen, indicating a common infection-driven response. When comparing microfilaria (mf)-positive and mf-negative patients, there were no significant differences in spontaneous cytokine nor in parasite-driven IL-10, IL-22, or IL-28a production. In marked contrast, mf-positive individuals had significantly increased filarial antigen-driven IL-24 and IL-19 compared to mf-negative subjects. mf-positive patients also demonstrated significantly higher frequencies of T cells producing IL-19 in comparison to mf-negative patients. T-cell expression of IL-19 and IL-24 was positively regulated by IL-10 and IL-1β. IL-24 production was also regulated by IL-37.

Conclusion. These data provide an important link between IL-10 and its related family members IL-19 and IL-24 in the modulation of the immune response in human filarial infections.

Clinical Trials Registration. **NCT00001230.**

Keywords. loiasis; microfilariae; IL-10 superfamily members; T-cell responses; cytokine regulation.

Patent chronic filarial infections are characterized by T-cell hyporesponsiveness associated with elevated levels of serum interleukin-10 (IL-10) and increased frequencies of IL-10–producing $CD4^+$ T cells [1]. This shift to a regulatory immune milieu leads to the suppression of T-cell proliferation and decreased interferon-γ (IFN-γ) and IL-2 production in response to filarial antigens as well as to bystander antigens [2–7]. IL-10 has been the cytokine of interest when evaluating the immune response during filarial infections. However, IL-10 belongs to a superfamily of cytokines that possess genetic similarities [8]. This grouping of cytokines includes IL-10, IL-19, IL-20, IL-22, IL-24, IL-28a, IL-28b, and IL-29. This extended family is highly pleiotropic and its individual members mediate diverse functions ranging from immune suppression to enhanced immunity to certain pathogens [8]. In the context of filarial infections, IL-19 and IL-24 are highly expressed during active bancroftian lymphatic filariasis (LF) and are associated with the modulation of T-cell responses during infection [9, 10]. T cells and myeloid cells are the primary source of IL-19 and IL-24 [11].

Almost all of the concepts relating clinical status (and patency) to the modulation of the cell response in filarial infection have been generated in the context of LF. However, another blood-borne filarial infection, *Loa loa* [12] has quite similar clinical spectra with the majority of chronic infections being subclinical and associated with high levels of circulating microfilariae (mf). Symptoms of *L. loa* infection (ie, Calabar swelling, urticaria, and myalgias) typically occur most frequently in patients without circulating mf [13–16]. Despite the parallels with LF, the nature of the immune response in *L. loa* infection has been largely understudied, though more attention to *L. loa* has been paid recently because of *Loa*-associated severe life-threatening adverse events following inadvertent ivermectin administration during mass drug administration campaigns for onchocerciasis elimination [17].

The present study provides novel information pertaining to a patient cohort with loiasis that has previously never been evaluated immunologically. Here, we show that IL-10 and its superfamily members IL-19 and IL-24 play a significant role as modulators of the immune response in loiasis and that it appears to be driven by mf. Moreover, IL-19 and IL-24 are positively regulated by IL-10 and IL-1β; additionally, IL-37 has an important regulatory effect on IL-24 production. Together, our

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data suggest that IL-19 and IL-24 are part of an intricate cascade of regulatory cytokines seen in filarial infections.

METHODS

Ethics Statement

Blood products used in this study were collected from *L. loa* infected patients under a protocol (NCT00001230) approved by the institutional review board of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, and informed written consent was obtained from all subjects.

Study Population

All patients were examined at the National Institutes of Health. The cohort consisted of 3 patients who had lived most of their lives in a region endemic for *L. loa* and 28 expatriates. None of the subjects had received anthelmintic/antifilarial treatment at the time of this study. Demographic and clinical information pertaining to the presence of mf in the circulation is provided in Table 1. This cohort was a small subset of subjects whose clinical details (signs and symptoms) have been previously described [13].

Isolation of PBMCs and In Vitro Cell Culture

Fresh peripheral blood mononuclear cells (PBMCs) isolated from whole blood samples from each patient prior to therapy were cultured in media alone or in the presence of either an extract of *Brugia malayi* adult antigen (BmA 5μg/mL) or 2 nonparasite control antigens tetanus toxoid (TT) and streptolysin O (SLO). Cells were incubated at 37°C, 5% CO_2, and supernatants were collected on days 1, 2, and 5 and stored at −80°C until used.

Luminex

The culture supernatants were assessed using a human Th17 panel customized for 14 analytes: IFN-γ, IL-10, IL-13, IL-17A, IL-22, IL-9, IL-2, IL-21, IL-4, IL-23, IL-5, IL-27, TNF-α, and IL-28α; and a cytokine/chemokine panel customized for 3 analytes: IL-19, IL-24, and IL-37 (EMD Millipore). The assay was performed according to the manufacturer's instructions, and samples were analyzed using a Bio-Plex 200 (Bio-Rad).

Flow Cytometry

Cryopreserved PBMCs from the same loiasis patients were thawed, washed, and counted. Cells were cultured in complete RPMI-1640 (10% fetal bovine serum) at 1×10^6 cells/mL in media alone or in the presence of either BmA (10 μg/mL) or a nonparasite control antigen SLO (1:100 final concentration; Difco). Cells were incubated at 37°C, 5% CO_2 for 12 hours. Brefeldin A and monensin were added 1 hour into the incubation at a final concentration of 5 μg/mL each.

For cytokine addition experiments, 40 ng/mL of recombinant human IL-10, IL-1β, IL-17, or IL-37 (R&D Systems) was cultured with patient PBMCs for 24 hours at 37°C, 5% CO_2. Brefeldin A and monensin were added for the final 8 hours of incubation. For cytokine neutralization experiments, patient PBMCs were cultured in the presence of isotype control antibody, anti-IL-10, anti-IL-1β, anti-IL-17, or anti-IL-37 (all at 5 μg/mL; R&D Systems) for 15 hours at 37°C, 5% CO_2. BmA (10 μg/mL) was then added and cultured for an additional 9 hours. Brefeldin A and monensin were added for the final 8 hours of incubation. Additionally, culture supernatants were collected 24 hours after BmA addition.

All samples were stained with LIVE/DEAD fixable blue stain (Invitrogen) for 15 minutes at room temperature. PBMC samples were stained with antibodies to CD3 (SK7; eBioscience), CD4 (RPA-T4; eBioscience), and CD8a (OKT8; eBioscience) for 30 minutes at room temperature. After fixation and permeabilization, intracellular staining was performed using antibodies to IL-19 (152112; R&D Systems) and IL-24 (polyclonal goat IgG; R&D Systems) for 30 minutes at 4°C. Detection of IL-24 required a second staining step with a conjugated donkey anti-goat IgG antibody (R&D Systems). Data were acquired using an LSRFortessa flow cytometer (BD Bioseceinces) and analyzed using FlowJo 10.5.0 software (Tree Star).

Enzyme-Linked Immunosorbent Assay

Concentration of IL-24 in PBMC culture supernatants was assessed using the human IL-24 DuoSet enzyme-linked immunosorbent assay (ELISA; R&D Systems).

Statistical Analysis

Paired samples were analyzed using the Wilcoxin test, and the Mann-Whitney test (nonparametric test) was used to compare different groups. The geometric mean was used as a measure of central tendency. For correlation analyses, Spearman rank correlations were calculated. The software GraphPad Prism 7 was used for all statistical analysis. *P* values less than .05 were considered significant.

Table 1. Characteristics of the Study Population

Characteristic	Microfilariae **Negative** (n = 22)	Microfilariae **Positive** (n = 9)
Median age, y (range)	27 (10–48)	31 (24–57)
Sex, male/female	14/8	6/3
Median microfilariae/mL (range)	0	18 (1–650)
Endemic/expatriate	0/22	3/6

RESULTS

Filarial Antigen Induces IL-10, Th2, and Th9 Cytokines in Loiasis Patients

PBMCs were isolated from 31 patients with confirmed *L. loa* infection. Upon isolation, the cells were placed in culture and stimulated with filarial antigen for 2 days. Compared to their respective baseline levels, loiasis patients had statistically significant increases in levels of IL-10 (*P* = .0414), IL-4 (*P* = .0094),

IL-5 ($P < .0001$), IL-13 ($P < .0001$), and IL-9 ($P = .0016$) upon filarial antigen stimulation (Figure 1A). These increased cytokine levels were parasite specific as stimulation with an unrelated antigen, TT, did not induce the production of these same cytokines (Figure 1B). IL-5 was the exception as there was significant induction following stimulation with TT ($P = .0312$). These data indicate that filarial parasite antigen drives the production of IL-10 as well as T helper 2 (Th2) and Th9 cytokines during *L. loa* infection. We next assessed the induction by filarial antigen of the IL-10 superfamily members, IL-19, IL-22, IL-24, and IL-28a in these patients. Filarial antigen stimulation did not induce the production of these cytokines (Supplementary Figure 1).

Because mf status can be associated with differential antigen-specific immune-responsiveness, we assessed the differences in IL-10 superfamily cytokine production in both mf-positive (n = 9) and mf-negative (n = 22) patients. There were no statistically significant differences in baseline secretion levels for any of the IL-10 superfamily members, IL-10 ($P = .4278$), IL-19 ($P = .2936$), IL-22 ($P = .0565$), IL-24 ($P = .1452$), and IL-28a ($P = .2634$) between mf-positive and mf-negative patients (Figure 2A). Therefore, infection status per se fails to alter the production of these specific members of the IL-10 superfamily. After stimulation with filarial antigen, the induction of IL-22 ($P = .5855$) and IL-28a ($P = .3717$) levels did not differ between mf-positive and mf-negative patients (Figure 2B). In marked contrast, we observed that the inductions of IL-19 ($P = .0316$) and IL-24 ($P = .0088$) were significantly greater in mf-positive patients compared to mf-negative patients following parasite antigen stimulation (Figure 2B). For IL-24, this observation proved to be parasite specific as stimulations with the unrelated antigens SLO ($P = .1733$) and TT ($P = .2057$) did not result in significant cytokine induction differences between the mf-positive and mf-negative groups (Supplementary Figure 2B). For IL-19, stimulation with SLO did not result in significant differences between mf-positive and mf-negative patients ($P = .3698$) (Supplementary Figure 2A). However, following TT stimulation, IL-19 induction levels were significantly greater in mf-positive patients ($P = .0048$) (Supplementary Figure 2A). These observations indicate that mf status influences the magnitude of IL-19 and IL-24 induction following specific antigen exposure.

Frequencies of IL-19–Producing T Cells Is Significantly Increased in Microfilaria-Positive Patients

Cryopreserved PBMCs from 26 of the original 31 loiasis patients (mf positive = 9 and mf negative = 17) were thawed and assessed by flow cytometry (Supplementary Figure 3). At baseline, there were no significant differences in the frequencies of $CD4^+$ and $CD8^+$ T cells producing IL-19 between mf-positive and mf-negative patients ($P = .7416$ and $P = .4910$, respectively) (Supplementary Figure 4A and 4B). However, following filarial antigen stimulation, the net frequencies of $CD4^+$ as well as $CD8^+$ cells producing IL-19 were significantly higher in mf-positive patients compared to mf-negative patients ($P = .0004$ and $P = .0243$, respectively) (Figure 3A and 3B). Therefore, IL-19 production by $CD4^+$ and $CD8^+$ T cells following parasite antigen stimulation is greater in those with patent infection. Stimulation with the unrelated antigen SLO resulted in similar frequencies of $CD4^+IL\text{-}19^+$ as well as $CD8^+IL\text{-}19^+$ ($P = .1599$ and $P = .0981$, respectively) T cells between mf-positive and mf-negative

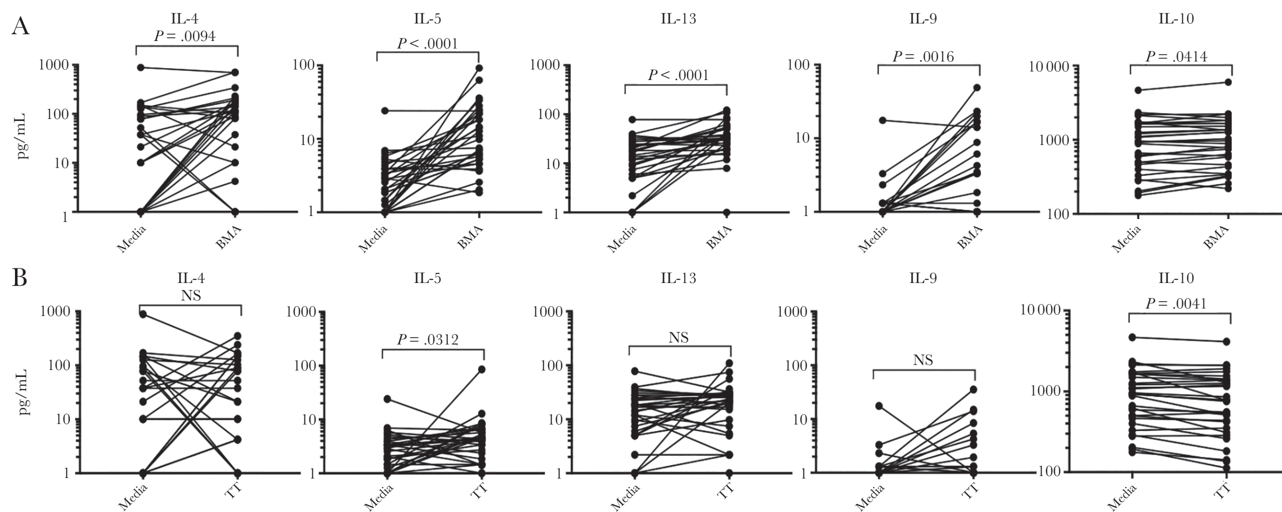

Figure 1. Cytokine levels in loiasis patients in response to antigen stimulation. Freshly isolated PBMCs from 31 loiasis patients were cultured for 2 days at 37°C, 5% CO_2 in media alone or in the presence of (*A*) BmA or (*B*) TT. Supernatants were analyzed by different Luminex panels and we assessed Th2, Th9, and regulatory cytokines such as IL-4, IL-5, IL-13, IL-9, and IL-10. NS, $P > .05$. Abbreviations: BmA, *Brugia malayi* adult antigen; IL, interleukin; NS, nonsignificant; PBMC, peripheral blood mononuclear cell; Th, T helper cell; TT, tetanus toxoid.

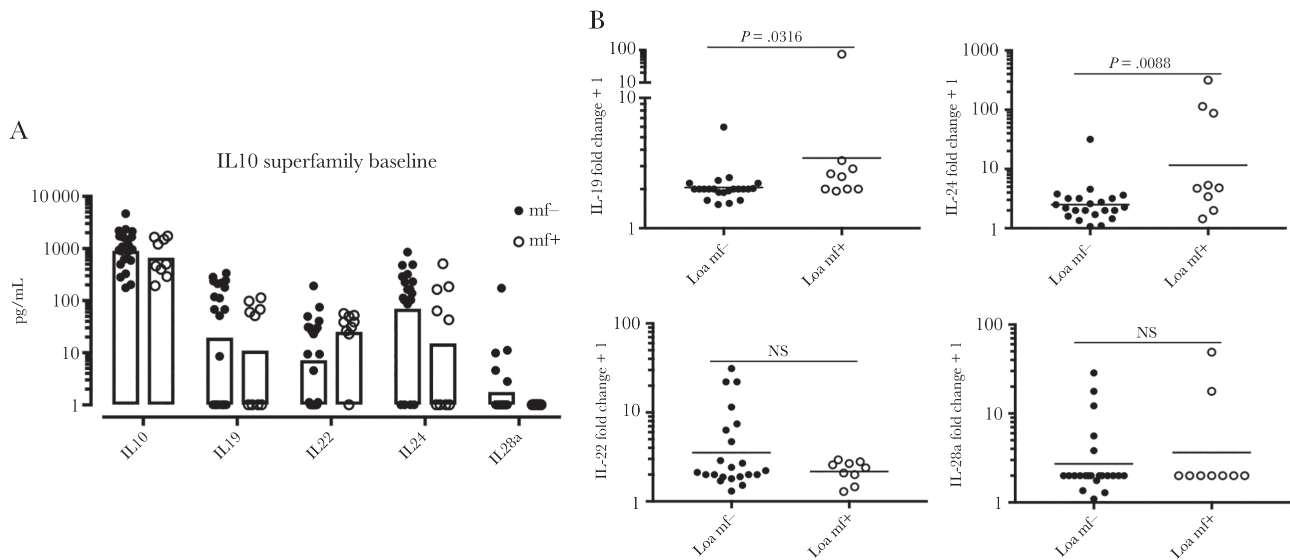

Figure 2. Spontaneous and parasite-driven IL-10 superfamily member cytokine production. Freshly isolated PBMCs from 31 loiasis patients (mf negative = 22, mf positive = 9) were cultured for 2 days at 37°C, 5% CO_2 in media alone or in the presence of BmA. Supernatants were collected and analyzed by Luminex. *A*, Spontaneous production of IL-10 superfamily member cytokines was quantified. *B*, The fold changes of IL-19, IL-24, IL-22, and IL-28a were calculated to compare filarial antigen stimulated conditions to baseline. NS, $P > .05$. Abbreviations: BmA, *Brugia malayi* adult antigen; IL, interleukin; Loa mf –, *Loa loa* microfilaria negative; Loa mf+, *Loa loa* microfilaria positive; NS, nonsignificant; PBMC, peripheral blood mononuclear cell.

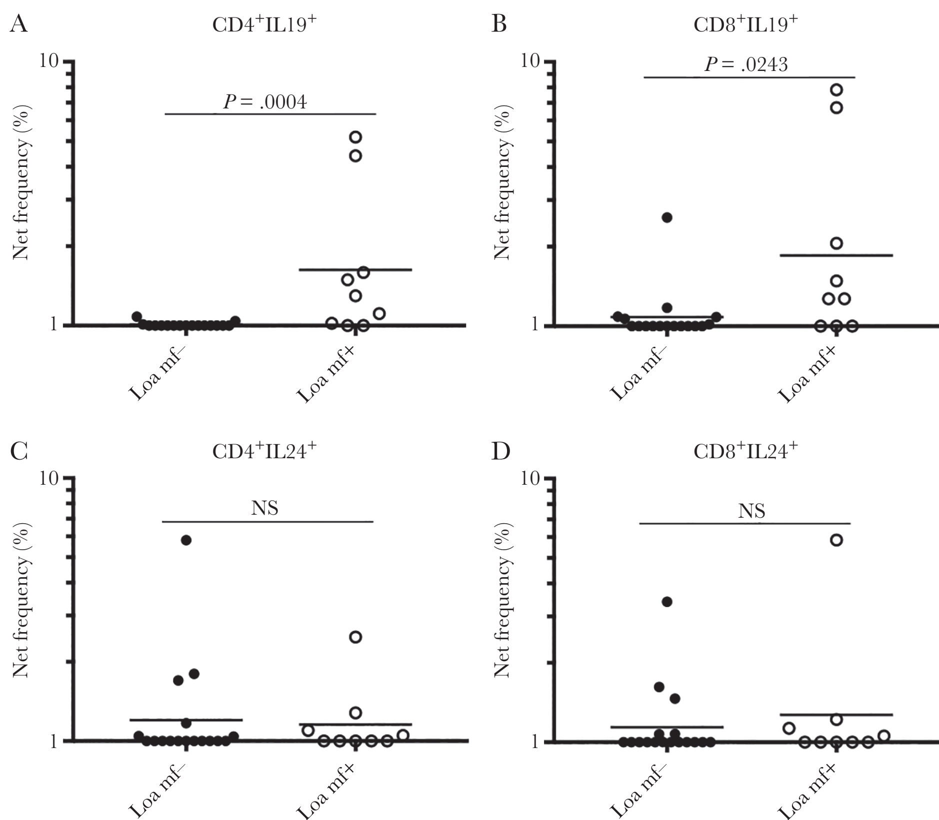

Figure 3. Net frequencies of T cells expressing IL-19 and IL-24 in response to parasite antigen. PBMCs from loiasis patients (mf negative = 17, mf positive = 9) were cultured in media alone or in the presence of filarial parasite antigen. The cells were then assessed by flow cytometry to determine the frequencies of parasite-specific (*A*) $CD4^+IL\text{-}19^+$, (*B*) $CD8^+IL\text{-}19^+$, (*C*) $CD4^+IL\text{-}24^+$, and (*D*) $CD8^+IL\text{-}24^+$ cells. The samples were gated on singlet/lymphocytes/live/$CD3^+$ cells. NS, $P > .05$. Abbreviations: IL, interleukin; Loa mf –, *Loa loa* microfilaria negative; Loa mf+, *Loa loa* microfilaria positive; NS, nonsignificant; PBMC, peripheral blood mononuclear cell.

patients (Supplementary Figure 5A), indicating that the increase seen in mf-positive patients is parasite specific.

The same analysis was conducted to characterize the IL-24 T-cell response. At baseline, there were no statistically significant differences in CD8$^+$IL-24$^+$ frequencies between mf-positive and mf-negative patients (P = .2000) (Supplementary Figure 4D). However, the baseline frequency of CD4$^+$IL-24$^+$ T cells in mf-negative patients was significantly higher than that observed in mf-positive patients (P = .0336) (Supplementary Figure 4C). Following parasite antigen stimulation, there were no statistically significant differences between either CD4$^+$ or CD8$^+$ T cells producing IL-24 when comparing mf-negative and mf-positive patients (P = .6864 and P = .5552, respectively) (Figure 3C and 3D). This was also true when observing frequencies of CD4$^+$IL-24$^+$ and CD8$^+$IL-24$^+$ cells following SLO stimulation (P = .8176 and P = .8776, respectively) (Supplementary Figure 5B). These observations suggest that upon filarial antigen stimulation, the frequencies of both CD4$^+$ and CD8$^+$ T cells producing IL-24 are similar between mf-negative and mf-positive patients.

IL-19 and IL-24 Regulation by IL-10, IL-1β, and IL-37

We next sought to investigate the cytokine network regulating the production of IL-19 and IL-24. Through correlation matrices, IL-19 concentrations correlated positively with concentrations of IL-17a (P = .0063; r = 0.487) and IL-27 (P = .0158; r = 0.4368) (Figure 4A and 4B). IL-24 concentrations correlated positively with concentrations of IL-5 (P = .0195; r = 0.4174) and IL-37 (P = .0241; r = 0.6303) (Figure 4C and 4D).

To investigate the role of IL-10, IL-1β [9], and IL-37 in expression patterns of T cells producing IL-19 and IL-24, we added these recombinant cytokines to PBMC cultures. Although not statistically significant, the addition of IL-10 resulted in an increased frequency of CD8$^+$ T cells producing IL-19 (P = .0625) (Figure 5A). The addition of IL-1β to cultures resulted in a statistically significant increase in the frequency of CD4$^+$IL-19$^+$ T cells (P = .0156) and a nonsignificant trend in CD8$^+$IL-19$^+$ T cells (P = .0781) (Figure 5A). The addition of IL-37 had no effect on the frequency of either CD4$^+$ or CD8$^+$ T cells producing IL-19 (P = .3828 and P = .3125, respectively) (Figure 5A). These observations suggest that IL-1β and IL-10 positively regulate the expression of IL-19 by T cells in patients infected with *L. loa*. Similar regulation patterns were observed for IL-24. The frequency of CD4$^+$ T cells expressing IL-24 was significantly increased upon the addition of IL-10, IL-1β, or IL-37 (P = .0007, P = .0327, and P = .0166, respectively) (Figure 5B). IL-10, IL-1β, or IL-37 also increased the frequency of CD8$^+$IL-24$^+$ T cells (P = .0010, P = .0134, and P = .0266, respectively) (Figure 5B). These data indicate that IL-24 expression by both CD4$^+$ and CD8$^+$ T cells in loiasis patients is positively regulated by IL-10, IL-1β, and IL-37.

To further understand the role played by IL-10, IL-1β, and IL-37 in the regulation of antigen driven IL-19 and IL-24,

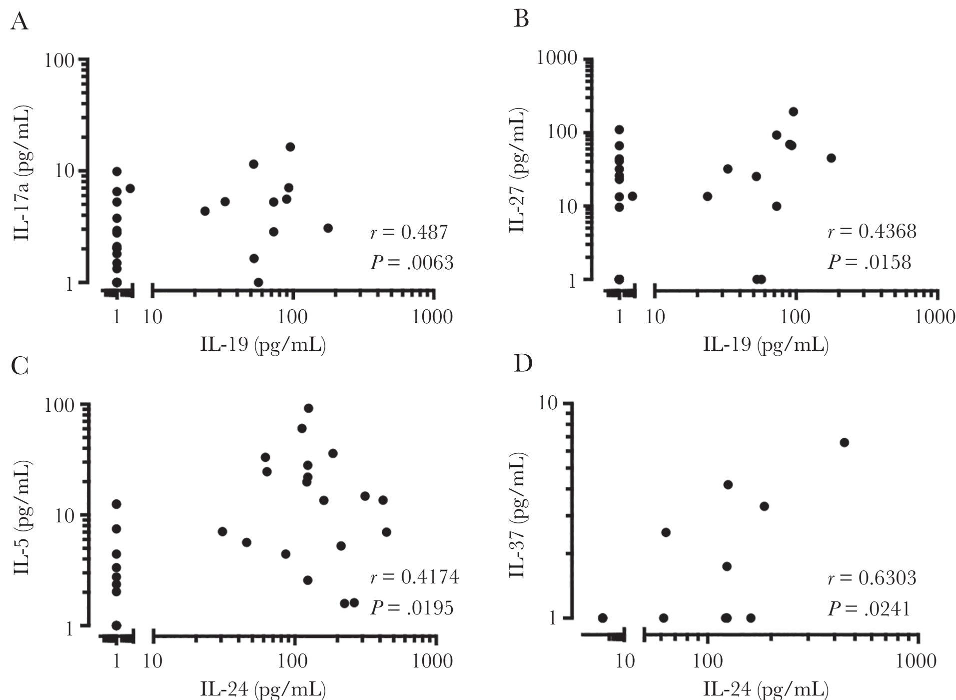

Figure 4. *A–D*, Cytokine correlations in loiasis patients. Freshly isolated PBMCs from 31 loiasis patients were cultured for 2 days at 37°C, 5% CO_2 in the presence of filarial antigen. Supernatants were assessed by different Luminex panels. Spearman correlations were calculated for the different sets of cytokines. Abbreviations: IL, interleukin; PBMC, peripheral blood mononuclear cell.

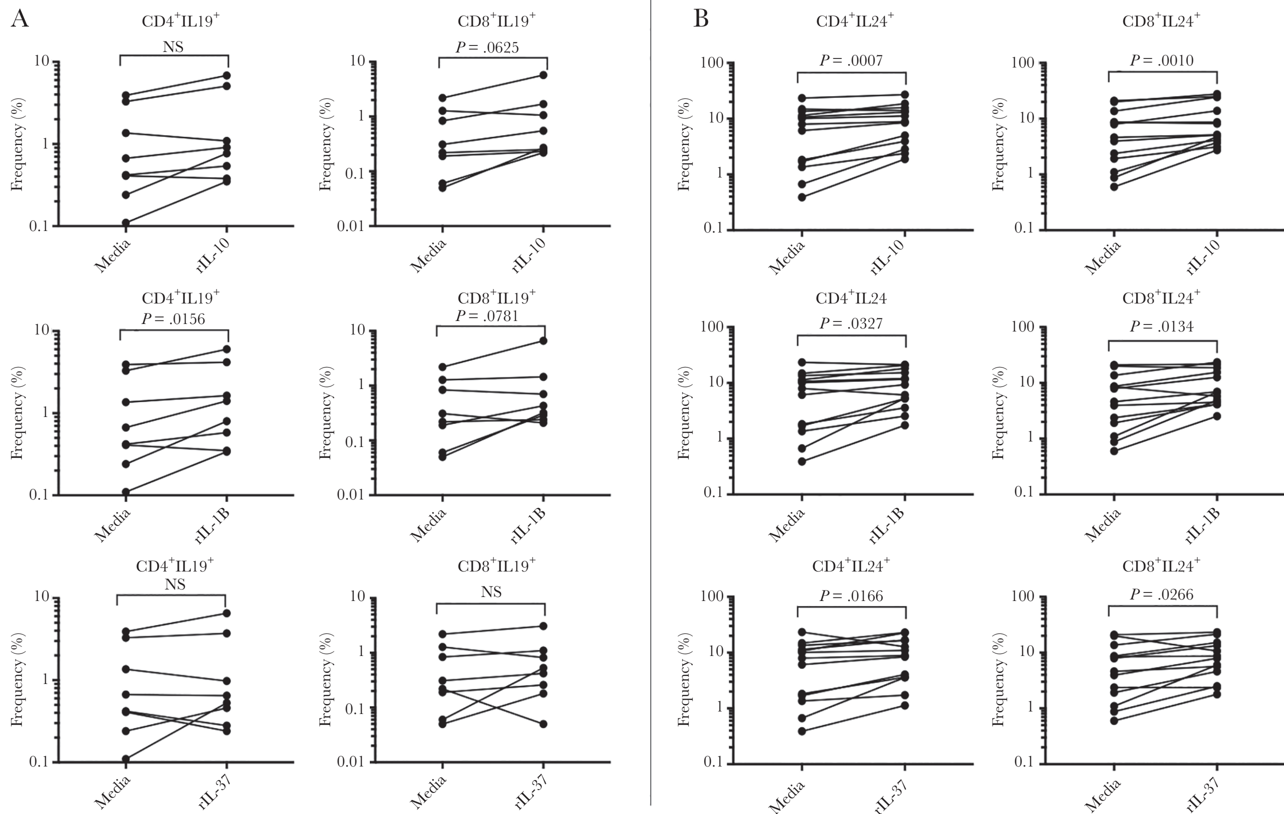

Figure 5. Frequencies of T cells producing IL-19 and IL-24 upon the addition of recombinant cytokine. PBMCs from loiasis patients were cultured in media alone or in the presence of 40 ng/mL of recombinant antigen (rIL-10, rIL-1β, or rIL-37) for 24 hours. The cells were then assessed by flow cytometry to determine the frequencies of (*A*) CD4+IL-19+ and CD8+IL-19+ (n = 8 for each subset), as well as (*B*) CD4+IL-24+ and CD8+IL-24+ (n = 13 for each). The samples were gated on singlet/lymphocytes/live/CD3+ cells. NS, *P*> .05. Abbreviations: IL, interleukin; NS, nonsignificant; PBMC, peripheral blood mononuclear cell.

we used neutralizing antibodies to each of these cytokines at the time of antigen stimulation. The addition of anti-IL-10 or anti-IL-1β did not alter the frequencies of either CD4+IL-19+ (*P* = .1250 and *P* = .6250, respectively) or CD8+IL-19+ (*P* > .9999, and *P* = .8125, respectively) T cells in response to filarial antigen stimulation (Supplementary Figure 6A). Similarly, the frequencies of CD4+IL-24+ (*P* = .4316 and *P* = .9219) and CD8+IL-24+ (*P* = .3750 and *P* = .9219) T cells did not change upon the addition of anti-IL-10 or anti-IL-1β (Supplementary Figure 6B). However, culturing patient PBMCs with anti-IL-37 prior to filarial antigen stimulation significantly decreased the frequencies of both CD4+IL-24+ (*P* = .0488) and CD8+IL-24+ (*P* = .0195) T cells compared to the isotype antibody control (Figure 6A and 6B). These data indicate that IL-37 is needed to promote IL-24 production following filarial antigen stimulation.

We also assessed the concentration of IL-24 protein after cytokine neutralization. Neutralizing IL-10, L-1β, or IL-37 led to a marked decrease in IL-24 secretion levels (*P* = .0156, *P* = .0312, and *P* = .0156, respectively) (Figure 6C). These results provide further evidence supporting a regulatory role for IL-37, and to a lesser extent IL-10 and IL-1β, in the production of IL-24.

DISCUSSION

The modified Th2/regulatory profile associated with human filarial infections is believed to be necessary for the maintenance of a chronic infection, often with high parasite densities [1–7, 18]. IL-10 has long been the leading candidate to mediate such regulation. However, recently, other members of the IL-10 superfamily have been shown to have regulatory effects in LF [9, 10]. In the current study, we focused on the role and regulation of IL-10 superfamily members in the context of *L. loa* infection with an emphasis on the role played by mf.

The prototypical immune response during most filarial infections involves increased production of IL-10 as well as Th2/Th9 cytokines [18]. These findings, not surprisingly, were recapitulated in the present study. Disease manifestation during *L. loa* infection is greatly influenced by the presence of mf in the peripheral blood [13–16]. Mf-negative patients are likely to have a higher prevalence of allergic symptoms [13, 19, 20]. In contrast, mf-positive patients are more likely to be clinically asymptomatic. In the absence of antigen stimulation, mf-negative and mf-positive loiasis patients produce the same levels of the IL-10 superfamily cytokines IL-10, IL-19, IL-22, IL-24, and IL-28a. However, upon filarial antigen stimulation, there were greater

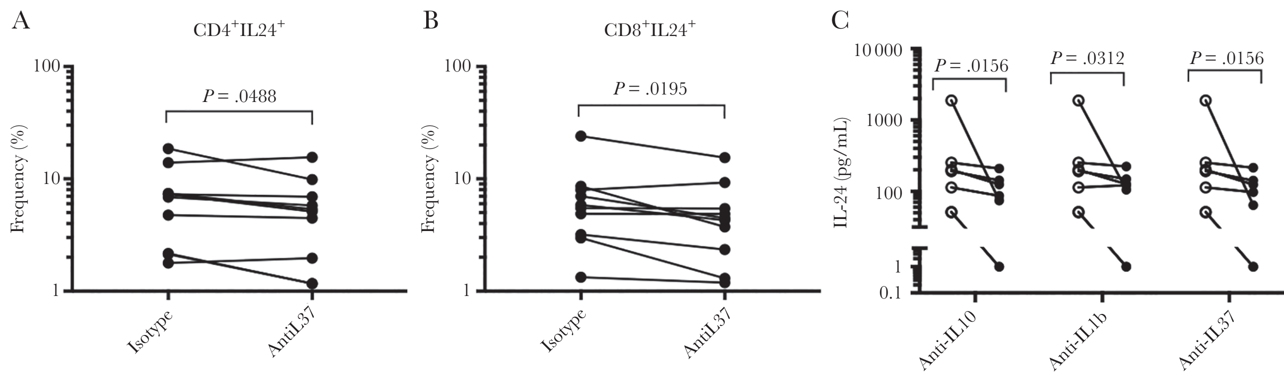

Figure 6. IL-24 production upon neutralization of certain cytokines. PBMCs from loiasis patients were cultured in the presence of isotype antibody or neutralizing antibody (anti-IL-10, anti-IL-1β, or anti-IL-37) for 15 hours. The cells were then stimulated with parasite antigen. The cells were assessed by flow cytometry to determine the frequencies of (*A*) $CD4^+IL\text{-}24^+$ (n = 10) and (*B*) $CD8^+IL\text{-}24^+$ (n = 10). The samples were gated on singlet/lymphocytes/live/$CD3^+$ cells. NS, $P > .05$. *C*, Supernatants were collected from patient PBMC cultures and concentrations of IL-24 were assessed by ELISA (n = 7). Abbreviations: ELISA, enzyme-linked immunosorbent assay; IL, interleukin; NS, nonsignificant; PBMC, peripheral blood mononuclear cell.

increases in IL-19 and IL-24 in mf-positive patients compared to mf-negative ones. These results suggest an association between patent *L. loa* infection and the parasite-driven production of IL-19 and IL-24, findings that have parallels in studies of a different filarial infection, *Wuchereria bancrofti*, during which these cytokines were postulated to mediate protection against lymphatic pathology [9, 10]. Although most of the literature for IL-24 focuses on its antitumor properties, a potential regulatory role for this cytokine in infectious diseases was revealed using a *Staphylococcus aureus* model, where both IL-19 and IL-24 were shown to suppress IL-1β and IL-17 [21]. In several disease models, IL-19 has demonstrated strong regulatory properties. For instance, IL-19 was shown to inhibit experimental atherosclerosis through alteration of proinflammatory genes [22]. Furthermore, in patients undergoing coronary artery bypass, IL-19 reduced T-cell responses while promoting the regulatory functions of $CD4^+$ T cells [23]. These observations support regulatory roles for IL-19 and IL-24, findings seen in the present study.

T cells are critical in all aspects of filarial infections [18, 24–26]. Chronic filarial infections are characterized by a modified Th2 response and an IL-10–dominated regulatory environment [18]. Alternatively, alterations or improper regulation of T-cell numbers and effector function are associated with immune-mediated pathology in LF [18]. Due to the critical importance of T cells during filarial infections, we chose to expand our investigation to include a characterization of T-cell expression patterns of IL-19 and IL-24. Although the antigen-specific T-cell expression patterns for IL-24 were similar between mf-positive and mf-negative individuals, there were expanded frequencies of both $CD4^+19^+$ and $CD8^+19^+$ T cells in mf-positive patients following filarial antigen stimulation. This increase in IL-19–producing T cells observed in patent infection could potentially indicate a role for this cytokine in mediating protection against pathology, as mf-positive individuals are more likely to be asymptomatic.

The cytokine networks regulating members of the IL-10 superfamily still require investigation. It has been suggested that IL-19 is a component of the IL-23/IL-17 cascade in psoriasis [27]. Additionally, it has been shown that IL-19 can induce its own expression and can also be regulated by IL-10 [28]. Little is known about the regulation of IL-24. It has been shown that IL-1β stimulation induces the expression of IL-24 in keratinocytes as well as subepithelial myofibroblasts [29]. We have previously shown that expression of both IL-19 and IL-24 by T cells of patients with LF is regulated by IL-10, IL-1β, and IL-23 [15]. In the correlation analyses performed in the present study, there were significant positive correlations between IL-19 and both IL-17a and IL-27. The correlation with IL-17a is supported by information in the literature that suggests a role for IL-19 in the IL-23/IL-17 cascade [27]. A relationship between IL-19 and IL-27 is particularly of interest because IL-27 possesses anti-inflammatory properties, and it can induce IL-10 from T cells [30]. IL-24 concentrations positively correlated with IL-5 and IL-37. IL-24 is known to be selectively expressed in activated Th2 cells, thus suggesting a positive association with Th2 cytokines such as IL-5 [31]. As for IL-37, its potential regulation with IL-24 represents a novel finding. IL-37 has been described as an anti-inflammatory cytokine that subdues innate functions by decreasing proinflammatory cytokines and inhibiting inflammasome activation [32, 33]. Furthermore, it is capable of inhibiting Th2 and Th9 activation in a mouse model of allergic bronchopulmonary aspergillosis [33]. Finally, IL-37 has been shown to possess antitumor properties [34–36], thus making its correlation with IL-24, also a tumor suppressor molecule, particularly interesting.

We chose to investigate the roles of IL-10, IL-1β, and IL-37 in the regulation of IL-19 and IL-24 production. The addition

of rIL-1β resulted in significant increases in the frequencies of patient T cells producing IL-19. The addition of rIL-10 resulted in an increasing trend as well. However, neutralization of IL-10 or IL-1β did not diminish the frequencies of T cells producing IL-19 in response to filarial antigen stimulation. These observations suggest that, although IL-10 and IL-1β may belong to a network regulating IL-19 production by T cells during *L. loa* infection, other cytokines with redundant functions may compensate in their absence. The frequencies of both $CD4^+$ and $CD8^+$ T cells producing IL-24 were significantly increased upon the addition of rIL-10, rIL-1β, or rIL-37. Like IL-19, IL-24 production seems to be positively regulated by IL-10 and IL-1β. Additionally, IL-37 can also be included as a potential regulator of IL-24. Furthermore, neutralizing IL-37 resulted in diminished frequencies of both $CD4^+$ and $CD8^+$ T cells producing IL-24. Neutralizing IL-10, IL-1β, or IL-37 in patient PBMC cultures yielded significantly lower concentrations of IL-24. These data further support a regulatory role for IL-10, IL-1β, and IL-37 in the production of IL-24 by various cellular sources.

Overall, the results of the present study suggest that the IL-10 superfamily members IL-19 and IL-24 are differentially regulated and expressed during *L. loa* infection. We have demonstrated that mf status is clearly related to the marked increases in IL-19 and IL-24 levels following parasite antigen stimulation and that IL-37, IL-10, and IL-1β are important regulators of IL-24, and to a lesser extent IL-19, production in human filarial infections. Whether this regulation extends to IL-26, another IL-10 superfamily member that appears to underly some of the severe pathology seen in LF [9], awaits further study. Continued research on the expression and regulation of IL-10 superfamily cytokines in the context of filariasis, as well as other chronic infections and diseases, is necessary as it would expand our understanding of disease progression and reveal novel therapeutic avenues.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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