

Eosinophil-Associated Processes Underlie Differences in Clinical Presentation of Loiasis Between Temporary Residents and Those Indigenous to *Loa*-Endemic Areas

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Background. *Loa loa* has emerged as an important public health problem due to the occurrence of immune-mediated severe posttreatment reactions following ivermectin distribution. Also thought to be immune-mediated are the dramatic differences seen in clinical presentation between infected temporary residents (TR) and individuals native to endemic regions (END).

Methods. All patients diagnosed with loiasis at the National Institutes of Health between 1976 and 2012 were included. Patients enrolled in the study underwent a baseline clinical and laboratory evaluation and had serum collected and stored. Stored pretreatment serum was used to measure filaria-specific antibody responses, eosinophil-related cytokines, and eosinophil granule proteins.

Results. *Loa loa* infection in TR was characterized by the presence of Calabar swelling (in 82% of subjects), markedly elevated eosinophil counts, and increased filaria-specific immunoglobulin G (IgG) levels; these findings were thought to reflect an unmodulated immune response. In contrast, END showed strong evidence for immune tolerance to the parasite, with high levels of circulating microfilariae, few clinical symptoms, and diminished filaria-specific IgG. The striking elevation in eosinophil counts among the TR group was accompanied by increased eosinophil granule protein levels (associated with eosinophil activation and degranulation) as well as elevated levels of eosinophil-associated cytokines.

Conclusions. These data support the hypothesis that differing eosinophil-associated responses to the parasite may be responsible for the marked differences in clinical presentations between TR and END populations with loiasis.

Keywords. *Loa loa*; eosinophil; loiasis.

Loa loa is a filarial parasite that infects approximately 13 million individuals in Central and West Africa [1, 2]. Chronic infection with *L. loa* can lead to nephropathy

[3], cardiomyopathy [4], retinopathy [5], neuropsychiatric complications [6], lymphedema [7], and encephalopathy [8]. Loiasis is an important public health problem due to the occurrence of immune-mediated serious adverse events in some individuals with high circulating levels of *L. loa* microfilariae following exposure to the antiparasitic drugs diethylcarbamazine (DEC) and ivermectin [9, 10]. As ivermectin is used in mass drug administration programs for onchocerciasis and (in combination with either DEC or albendazole) for lymphatic filariasis control, these *Loa*-associated posttreatment reactions have halted or impeded mass drug administration programs [11].

Prior studies have found marked differences in the clinical presentation of loiasis between those who acquire the infection as temporary residents (TR) and

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individuals native to endemic regions (END). Similar to the serious adverse events following exposure to antiparasitic drugs, the differences in clinical presentation between these 2 groups are thought to be immune-mediated [12–14]. TR experience greater “allergic” symptoms, are more often amicrofilaremic, and have markedly increased eosinophil counts compared with END [12–14]. The exact immunologic mechanisms underlying these clinical syndromes are unknown. Additionally, whether the quantitative differences in eosinophil counts between the 2 groups are accompanied by further differences in eosinophil regulation, function, or activation and whether additional immune pathways are altered between these groups has not been well studied heretofore. Because both posttreatment reactions as well as the differing clinical presentations between the 2 groups are likely related to immunologic responses to parasite antigens, an improved understanding of the immunology of loiasis could provide new strategies to ameliorate symptoms and prevent posttreatment reactions in patients with *L. loa* infection.

In the present study, we aimed to explore the varied clinical presentations of loiasis and to evaluate the impact of alterations in immune function on these differing presentations. As such, we explored the basis for the differences between the TR and END populations infected with *L. loa* by assessing the clinical presentation, filaria-specific antibody responses, and eosinophil-related activation markers and cytokines in 186 patients with loiasis.

MATERIALS AND METHODS

Study Population

All patients seen by the Clinical Parasitology Section of the Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases (NIAID) between 1976 and 2012 with loiasis were included in this study. Loiasis was defined by the presence of blood microfilariae, microfilarial DNA by polymerase chain reaction (PCR) testing, an adult worm on biopsy, or the presence of either an eyeworm or Calabar swelling in individuals with a relevant exposure history and the presence of antifilarial antibodies [15].

Informed consent was obtained from all patients, and all studies were performed under protocols approved by the Institutional Review Board of the NIAID. Between 1988 and 2012, the study was conducted under the registered protocol NCT00001230. A subset of patients (n = 42) included in this study has been described previously [12–14].

Clinical Evaluation

All patients underwent a baseline evaluation that consisted of a history and physical examination; complete blood count; urinalysis; stool exams for parasite ova and larvae; blood filtration

for microfilaremia (between 10 AM and 2 PM); quantification of immunoglobulin G (IgG; normal range, 700–1500 mg/dL), immunoglobulin M (IgM; 60–300 mg/dL), immunoglobulin A (IgA; 60–400 mg/dL), and immunoglobulin E (IgE; 3–423 IU/mL); and filaria-specific IgG, IgG4, and IgE. Blood filtrations were performed using Nuclepore filtration (Pleasanton, California) [16] of 1 mL of whole blood. Patients with a history of residence/exposure in onchocerciasis-endemic areas underwent skin snips using a corneoscleral punch (Storz, St Louis, Missouri) and microscopic and/or PCR testing for *Onchocerca volvulus* [17]. Those with potential exposure to *Wuchereria bancrofti* had Nuclepore filtration performed on blood collected between 10 PM and 12 AM and/or circulating antigen testing using the TropBio enzyme-linked immunosorbent assay (ELISA; JCU Tropical Biotechnology Private Limited, Queensland, Australia) [18, 19]. Additional testing was performed as clinically indicated.

Immunoassays for Parasite-Specific IgG and IgG4

IgG and IgG4 antibody response to crude protein extracts of *Brugia malayi* adult antigen (BMA) is cross-reactive in serum samples of patients with all of the filarial pathogens as well as with some intestinal helminths [20]. Filaria-specific IgG (BMA-IgG) and IgG4 (BMA-IgG4) were measured by ELISA as described previously [15, 21]. Normal cutoffs for both assays were defined on the basis of a reference serum above the 99% confidence interval for antibody concentrations from 62 healthy North Americans. Serum levels of BMA-IgG >14 µg/mL and BMA-IgG4 >0 ng/mL are considered above the normal range.

Eosinophil Granule Proteins

Granule protein levels including eosinophil cationic protein (ECP), eosinophil-derived neurotoxin, and eosinophil peroxidase (EPO) were measured with a multiplexed immunoassay using Luminex xMAP technology (Luminex Corporation, Austin, Texas) performed exactly as described previously [22]. The limits of the assays were 1 µg/mL for ECP, 0.24 µg/mL for eosinophil-derived neurotoxin, and 0.43 µg/mL for EPO.

Cytokine Measurement

Serum cytokine levels were measured using a custom-made xMAP human cytokine magnetic bead kit (Luminex Corporation, Austin, Texas). This kit measured interleukin (IL) 3, eotaxin, IL-2, IL-4, IL-5, interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), IL-17, granulocyte macrophage colony-stimulating factor (GM-CSF), and IL-6.

Statistical Analysis

Unless stated otherwise, geometric means were used as measures of central tendency, Fisher exact test was used to compare categorical variables, and the Mann–Whitney *U* test was used

Table 1. Demographic and Parasitological Characteristics of Endemic Individuals and Temporary Residents With Loiasis

Characteristic	Endemic Individuals	Temporary Residents	Total
No. of patients	42	144	186
Age, y, median (IQR)	35 (29–41)	27 (25–30)	27 (25–34)
Sex, male/female, No.	23/19	83/61	106/80
Length of exposure to endemic area, wk, median (IQR)	NA	108 (104–144)	NA
Patients infected with pathogenic intestinal helminths ^a , No. (%)	7/37 (18.9)	26/133 (19.5)	33/170 (19.4)
Patients infected with other filarial parasites ^b , No. (%)	7/42 (16.7)	11/144 (7.6)	18/186 (9.7)

Abbreviations: IQR, interquartile range; NA, not applicable.

^a Of patients who had stool ova and parasites evaluated. The most common intestinal helminths among those with positive stool exams in both groups were *Trichuris trichiura* (20/33 [61%]) and *Ascaris lumbricoides* (10/33 [30%]).

^b Of the 18 individuals coinfecting with another filarial parasite, 12 were infected with *Onchocerca volvulus*, 5 were infected with *Mansonella perstans*, and 1 patient was infected with both *M. perstans* and *O. volvulus* in addition to *Loa loa*.

for continuous variables. All statistics were performed in Prism version 6 (GraphPad, San Diego, California). *P* values <.05 were considered statistically significant.

RESULTS

Demographic Information

Patient demographics are summarized in Table 1. All patients had been residents in areas of Africa endemic for *L. loa*. All but 9 of the 144 TR had an exposure time of >6 months in a *Loa*-endemic area. There were no significant differences between the groups in the proportion of individuals coinfecting with intestinal helminths or with other filarial parasites (Table 1).

Clinical Presentation

The initial presentation of *L. loa* infection differed dramatically between the 2 groups (Table 2). The characteristic clinical finding of loiasis, Calabar swellings (transient localized angioedema), was much more common in the TR group (118/144 [82%]) compared with the END group (21/42 [50%], *P* < .0001). Other symptoms, such as urticaria, were also more prominent among TR (38/144 [26%]) than END (2/42 [4.8%], *P* = .002; Table 2).

In contrast, the presence of eyeworm was significantly more frequent in the END group than in the TR group (30/42 [71%] vs 22/144 [15%], respectively, *P* < .0001), as was microfilaremia (31/42 [74%] vs 31/144 [22%], respectively, *P* < .0001). Furthermore, among those with microfilariae, microfilarial counts were also higher in the END group (geometric mean, 942.4 microfilariae/mL [95% confidence interval {CI}, 390–2280]) than in the TR group (geometric mean, 72.8 microfilariae/mL [95% CI, 22–240], *P* = .003; Table 2).

Table 2. Presentation, Clinical Symptoms, and Complications of Loiasis at Presentation

Symptom	Endemic Individuals, No. (%) (n = 42)	Temporary Resident, No. (%) (n = 144)	Total No. (%) (N = 186)	<i>P</i> Value
Calabar swelling	21 (50)	118 (81.9)	139 (74.7)	<.0001 ^a
Eyeworm	30 (71.4)	22 (15.3)	52 (28)	<.0001 ^a
Microfilariae positive	31 (73.8)	31 (21.5)	62 (33.3)	<.0001 ^a
Microfilarial count (in microfilaria-positive patients), geometric mean (95% CI)	942.4 (390–2280)	72.8 (22–240)	275.7 (124–612)	.003 ^b
Murmur	6 (14.3)	28 (19.4)	34 (18.3)	.51 ^a
Hematuria ^c	7 (16.7)	19 (13.2)	26 (14)	.6 ^a
Proteinuria ^d	7 (16.7)	8 (5.6)	15 (8.1)	.046 ^a
Limb swelling	2 (4.8)	13 (9.0)	15 (8.1)	.53 ^a
Pleural effusion	1 (2.4)	2 (1.4)	3 (1.6)	.5 ^a
Urticaria	2 (4.8)	38 (26.4)	40 (21.5)	.002 ^a
Cardiac symptoms ^e	10 (23.8)	13 (9.0)	23 (12.4)	.02 ^a
EKG abnormalities ^f	10/20 (50)	15/69 (22)	25/89 (28)	.02 ^a

Abbreviations: CI, confidence interval; EKG, electrocardiographic.

^a Fisher exact test examining differences between individuals native to endemic regions (END) and temporary residents (TR).

^b Mann–Whitney *U* examining differences between END and TR.

^c Three patients with urinary tract infection (UTI) excluded from analysis; 1 female patient excluded from analysis as was menstruating at time of exam.

^d Two patients with UTI excluded from analysis.

^e Cardiac symptoms included palpitations, chest pain, congestive heart failure symptoms (eg, orthopnea, leg edema), dyspnea on exertion, shortness of breath, and decreased exercise tolerance.

^f Of patients who had EKG evaluation. EKG abnormalities included nonspecific T-wave abnormalities, sinus bradycardia or sinus tachycardia, left ventricular hypertrophy, and right axis deviations.

Table 3. Baseline Laboratory Values

Laboratory Test	Endemic Individuals, GM (95% CI)	Temporary Residents, GM (95% CI)	Total GM (95% CI)	P Value ^a
AEC, cells/mL	669.9 (423–1061)	1532 (1252–1875)	1270 (1048–1539)	<.0001
WBC, $\times 10^3$ /mL	6.4 (5.7–7.2)	9.0 (8.5–9.5)	8.3 (7.9–8.8)	<.0001
Polyclonal IgE, IU/mL	684.7 (439–1068)	271.9 (197–376)	342.5 (261–449)	.003
Polyclonal IgG, mg/dL	1881 (1715–2063)	1168 (1085–1258)	1305 (1220–1396)	<.0001
BMA-specific IgG, μ g/mL	120.5 (69–212)	1013 (678–1512)	592 (406–862)	<.0001
BMA-specific IgG4, ng/mL	2526 (1547–4124)	2366 (1464–3825)	2413 (1683–3459)	.6
Eosinophil cationic protein, ng/mL	72 388 (32 206–162 705)	130 138 (78 384–216 065)	111 295 (72 912–169 884)	.15
Eosinophil-derived neurotoxin, ng/mL	738 (423–1287)	909.6 (556–1487)	861.1 (586.9–1263)	.16
Eosinophil peroxidase, ng/mL	1.9 (.3–12.1)	4.9 (1.8–13.2)	3.8 (1.6–9.0)	.5

Abbreviations: AEC, absolute eosinophil count; BMA, *Brugia malayi* adult antigen; CI, confidence interval; GM, geometric mean; IgE, immunoglobulin E; IgG, immunoglobulin G; WBC, white blood cell.

^a Mann–Whitney *U* test examining differences between individuals native to endemic regions and temporary residents.

Complications associated with *L. loa* infection were infrequent in both groups (Table 2). Complications associated with chronic infection were significantly more frequent in the END group compared with the TR group, such as proteinuria (7/42 [17%] vs 8/144 [6%], respectively, $P < .05$) and cardiac symptoms (10/42 [24%] vs 13/144 [9%], respectively, $P = .02$). The increase in cardiac symptoms among END was also accompanied by an increased rate of minor electrocardiographic (EKG) abnormalities in this group (10/20 [50%] vs 15/69 [22%] in TR, $P = .02$), which consisted predominately of non-specific T-wave abnormalities, left ventricular hypertrophy, sinus bradycardia, sinus tachycardia, and right axis deviations.

Hematologic Evaluation

Baseline laboratory evaluations are shown in Table 3. In the TR group, geometric mean white blood cell (WBC) counts (9.0×10^3 cells/ μ L) as well as the proportion of individuals with an elevated WBC count (54/144 [38%]) were both increased compared with END (geometric mean WBC, 6.4×10^3 cells/ μ L, $P < .0001$; 5/42 [12%] END had elevated WBC count, $P = .002$). Absolute eosinophil count (AEC) also differed dramatically between the groups. Almost all TR (120/143 [84%]) had a peripheral blood eosinophilia (defined as AEC ≥ 500), with a geometric mean of 1532 cells/mL (95% CI, 1252–1875). In contrast, only 64% of END (27/42, $P < .01$) had elevated AEC, and the geometric mean AEC among END (670 cells/mL [95% CI, 423–1061]) was 2-fold lower than in the TR group (1532 cells/mL, $P < .0001$). However, when individuals with elevated AEC (≥ 500 cells/mL) were excluded from the analysis, no difference in mean WBC counts (5.7×10^3 cells/ μ L [SD, 3.5] in END vs 6.3×10^3 cells/ μ L [SD, 1.7] in TR, $P = .08$) or the proportion of individuals with an elevated WBC count (0/23 in TR and 1/15 in END, $P = .4$) was seen between the groups.

Polyclonal and Antigen-Specific IgG and Polyclonal IgE Levels

END patients had an increase in polyclonal IgG (geometric mean, 1881 mg/dL [95% CI, 1715–2063] vs 1168 mg/dL [95% CI, 1085–1258] in TR, $P < .0001$) and IgE (geometric mean, 684.7 ng/mL [95% CI, 439–1068] vs 271.9 [95% CI, 197–376] in TR, $P = .003$; Table 3). Baseline polyclonal IgE levels were significantly correlated to AEC for both the TR ($P = .01$, $r = 0.2$) and END ($P < .01$, $r = 0.4$) groups.

Filaria-specific BMA IgG levels were significantly higher in TR (1013 μ g/mL [95% CI, 678–1512]) compared to END (121 μ g/mL [95% CI, 69–212], $P < .0001$). TR also had a significantly greater IgG-specific percentage (BMA-IgG/total IgG) compared with END (7.2% vs 0.6%, respectively, $P < .0001$). Mean BMA-specific IgG levels in both groups, as well as in the total population, were unrelated to the presence or absence of Calabar swelling ($P > .05$ for all groups). BMA-specific IgG levels were largely unrelated to the presence of circulating microfilariae, although for the TR group, filaria-specific IgG levels were higher in the microfilaria (MF)-negative group (1258 μ g/mL [95% CI, 778–2035]) than in the MF-positive group (585 μ g/mL [95% CI, 283–1212], $P = .04$).

Eosinophil Granule Proteins

The geometric mean levels of 3 eosinophil granule proteins at the time of presentation (Figure 1 and Table 3) were higher (although not statistically significantly) in the TR group than in the END group. Eosinophil-derived neurotoxin in the TR group and in the total patient group (but not in END) was significantly correlated with AEC at the time of presentation (TR: $r = .4$, $P = .02$; total patient group: $r = 0.34$, $P < .01$; Figure 1). Neither EPO nor ECP was significantly correlated with AEC.

Cytokine Levels

For each cytokine in the panel measured, geometric mean levels were higher in TR than in END (Figure 2). Pretreatment GM-CSF

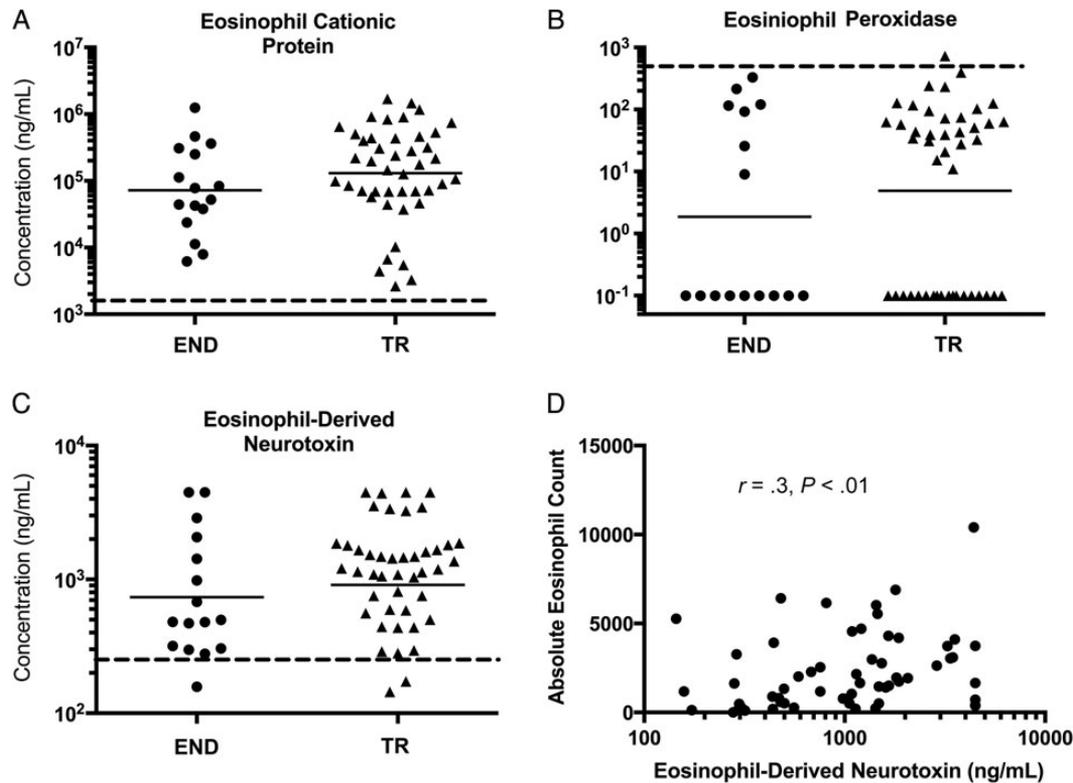


Figure 1. A–C, Pretreatment serum levels of eosinophil granule proteins. Each symbol represents an individual patient at presentation. The horizontal lines are the geometric mean. The dashed horizontal lines represent the limits of detection of the assay; values below these lines were calculated based on the standard curves. D, Correlation of serum eosinophil-derived neurotoxin (EDN) levels with absolute eosinophil count in the total patient group. Each dot represents an individual patient. Abbreviations: END, individuals native to endemic regions; TR, temporary residents.

and IL-5 levels were 5- to 10-fold higher in the TR group compared with END (geometric mean GM-CSF levels, 2.9 pg/mL [95% CI, 1.6–5.1] vs 0.3 pg/mL [95% CI, .1–.7], respectively, $P < .001$; geometric mean IL-5, 2.0 pg/mL [95% CI, 1.3–2.9] vs 0.5 pg/mL [95% CI, .2–1.2], respectively, $P < .01$). Eotaxin levels also differed significantly between groups (63 pg/mL [95% CI, 52–76] in TR vs 37.5 pg/mL [95% CI, 18.7–75.2] in END, $P = .05$). Cytokines related to Th2 (IL-4) and Th1 (IL-2 and IFN- γ) T-cell subset development were all significantly elevated in the TR group compared with END (geometric mean IL-4 level, 2 pg/mL [95% CI, 1.1–3.5] vs 0.3 pg/mL [95% CI, .1–.8], respectively, $P = .004$; geometric mean IFN- γ level, 3.3 pg/mL [95% CI, 1.5–7.3] vs 0.7 pg/mL [95% CI, .1–3.9], respectively, $P = .08$; geometric mean IL-2 level, 1.1 pg/mL [95% CI, .5–2.7] vs 0.1 pg/mL [95% CI, .07–.2], respectively, $P = .03$).

Only the levels of IL-5 were significantly correlated with the AEC in the TR group ($r = 0.3, P = .01$), the END group ($r = 0.7; P < .001$), and as a whole ($r = 0.4, P < .0001$). Cytokine levels did not correlate significantly with baseline IgE levels.

Cytokine levels did not differ significantly between those with and without Calabar swelling in the TR, END, or the entire

group for any of the assayed cytokines (all $P > .05$, data not shown). The impact of microfilaremia on cytokine levels was also examined (Figure 3). Amicrofilaremic patients demonstrated an increase in Th2-associated cytokine levels compared with microfilaremic patients (geometric mean IL-5, 2.2 pg/mL [95% CI, 1.4–3.4] vs 0.7 pg/mL [95% CI, .4–1.4], respectively, $P = .008$; geometric mean IL-4, 2.5 pg/mL [95% CI, 1.4–4.5] vs 0.5 pg/mL [95% CI, .2–1.1], respectively, $P = .001$). These amicrofilaremic patients also had an elevation in GM-CSF compared to those with microfilaremia (geometric mean, 3.1 pg/mL [95% CI, 1.6–5.8] vs 0.7 pg/mL [95% CI, .3–1.6], respectively, $P = .01$). However, Th1-associated cytokines (IL-2 and IFN- γ) and eotaxin did not differ significantly between those with and without microfilaremia.

DISCUSSION

A renewed interest has been seen in *L. loa* infection because of the serious adverse events in *L. loa*-infected patients following ivermectin administration. These serious adverse events are thought to be immune-mediated. Immune-mediated differences are also

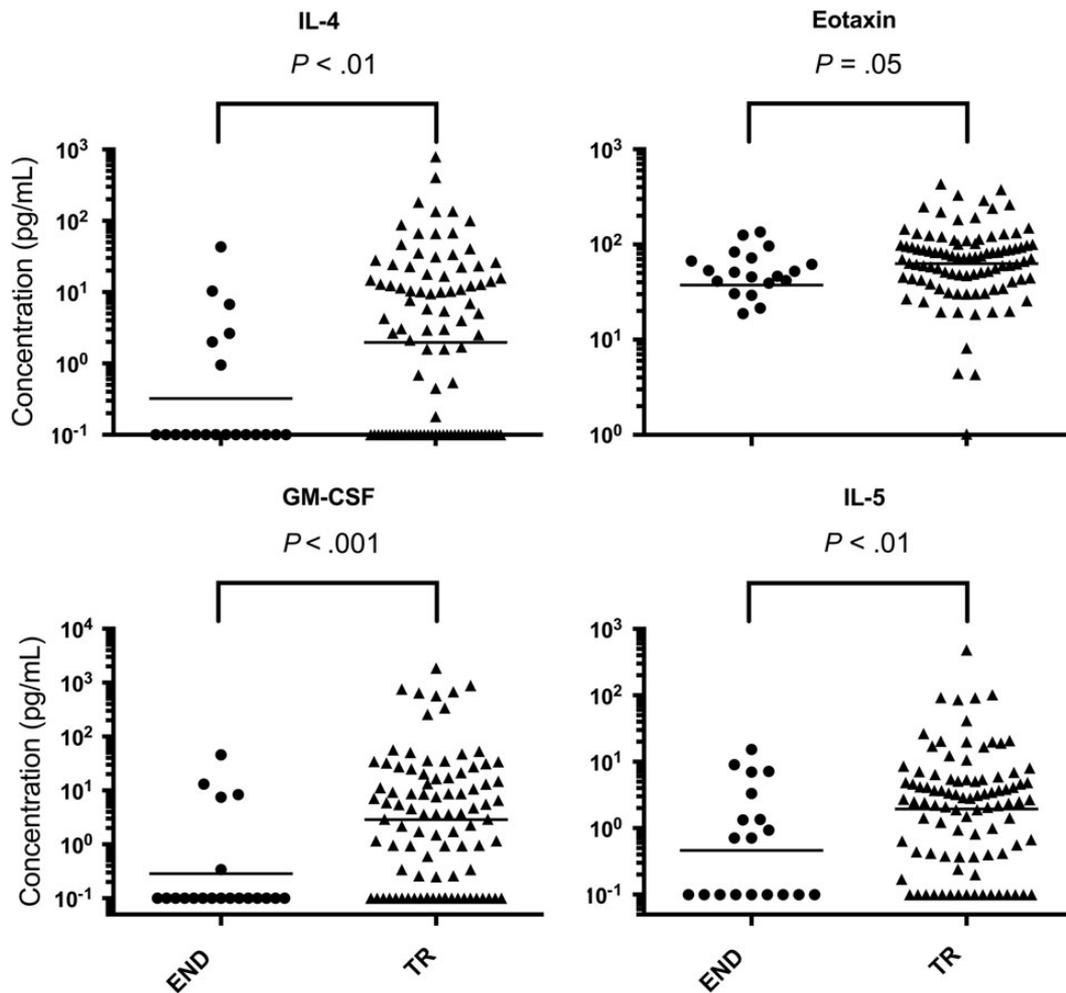


Figure 2. Serum cytokine levels in individuals native to endemic regions and temporary residents with loiasis. Each symbol represents an individual patient. Horizontal bars represent the geometric mean in each group. Abbreviations: END, individuals native to endemic regions; GM-CSF, granulocyte macrophage colony-stimulating factor; IL-4, interleukin 4; IL-5, interleukin 5; TR, temporary residents.

believed to underlie the dramatically different syndromes of loiasis experienced by TR compared to END [2, 12, 14, 23]. This study underscores the presence of a “hyperresponsive” syndrome among TR, a group previously shown to have had a higher prevalence of *Loa*-specific and “allergic” symptoms compared to the endemic study populations [12, 14, 24, 25]. END had a dampened clinical response and (although not demonstrated formally in the present study) immune-mediated tolerance to filarial antigens [12]. The differing rates of eyeworm between groups was not seen in earlier studies [12], and may be due to the END group presenting at a later stage of infection than TR or to increased adult worm burden in the END group.

Interestingly, minor cardiac abnormalities appeared to be prominent in loiasis, with a large proportion of patients positive for cardiac symptoms by history and/or the presence of a heart murmur or arrhythmia. Overall, cardiac symptoms and EKG

abnormalities both appeared to be most prevalent in the END group. Cardiac findings in loiasis have been attributed to endomyocardial fibrosis related to inflammation from eosinophil infiltration and degranulation. The true nature of this association is unknown, as only a single TR patient in the study had an endomyocardial biopsy [12, 14, 24–26]. It is notable that cardiac symptoms were more prevalent among the END group, which had lower average AEC. However, END likely had a greater duration of infection than TR, and therefore it is possible that the chronicity of eosinophilia rather than the degree of eosinophilia is the main risk factor for cardiomyopathy among loiasis patients. Additionally, the presence of common risk factors of cardiac disease was not assessed systematically.

With the finding of elevated eosinophil granule proteins in the TR group as well as the marked differences in eosinophil-related cytokines between the groups, it seems clear that

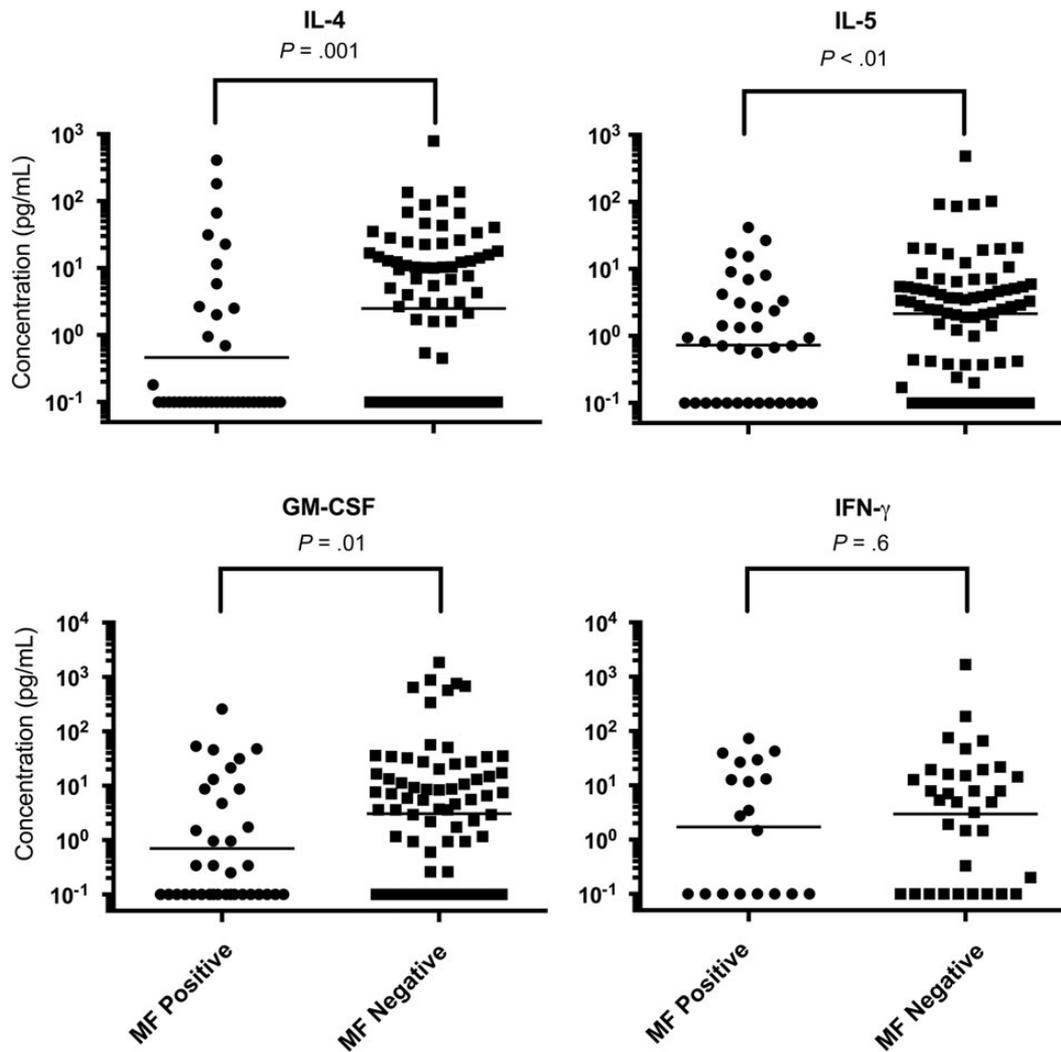


Figure 3. Cytokine levels in microfilaria-positive and -negative patients. Each symbol represents an individual patient. Horizontal bars represent the geometric mean in each group. Abbreviations: GM-CSF, granulocyte macrophage colony-stimulating factor; IFN- γ , interferon gamma; IL-4, interleukin 4; IL-5, interleukin 5; MF, microfilaria.

eosinophil regulation, activation, and recruitment differ dramatically between the 2 groups. The degree to which these differences contribute to the different clinical syndromes experienced by END and TR is unknown and merits further study.

The impact of microfilariae on immune response to infection has been well studied. Peripheral blood mononuclear cells from microfilaremic human donors have been found to have a dampened response to filarial antigen compared to those from amicrofilaremic individuals [27]. Additionally, studies in experimentally infected monkeys have shown that infected monkeys who become microfilaremic demonstrate declining cellular responsiveness, decreased cell proliferation, and diminished concentrations of IFN- γ , IL-2, IL-4, and IL-5 when microfilariae appear [28, 29]. The underlying mechanisms responsible for this downregulation of immune response is not clear, but may

be related to alterations in CD8⁺ cells [30], clonal deletion or clonal anergy of parasite-specific precursor T cells [31], or other factors.

Given the markedly increased rate of microfilaremia in the END group, these same decreases in cytokine responsiveness would be expected in this group. This was the case in the present study, but our results demonstrate additional immune alterations between groups that cannot be explained solely by differing rates of microfilaremia. As predicted, individuals in the END group were found to have significantly lower levels of both Th1 (IFN- γ and IL-2) and Th2 (IL-4 and IL-5) cytokines as well as lower levels of stimulators of eosinophil development (GM-CSF and IL-5) and recruitment (eotaxin). However, only the Th2-associated cytokines levels were diminished by microfilaremia. Although Th1-associated cytokines and eotaxin differed

between the END and TR groups, these cytokine levels did not vary between those with and those without microfilariae.

Differences in antibody production were also seen between groups. Parasite-specific IgG levels were significantly lower in the END group than in the TR group. Previous studies found a higher level of BMA-specific IgG in patients with Calabar swelling, suggesting that B-cell activation may contribute to the clinical hyperresponsiveness seen in TR [12]. However, this did not appear to be the case in the present study.

A limitation of the current study is the relatively small number of END subjects compared to TR; nevertheless, this study, to our knowledge represents the largest cohort of loiasis patients examined. Because the evaluation of all patients was performed at a state-of-the-art tertiary referral center, we did not include those END subjects studied abroad (eg, Benin, Cameroon) as they had not received the extremely detailed clinical and diagnostic evaluations that were provided to all subjects in this study. In addition, although it is possible that some of the subjects had cryptic non-*Loa* coinfections, it is unlikely given the extensive evaluation performed for other filarial and related helminth parasites. We acknowledge that eosinophil counts have been shown to be lower in subjects infected with malaria, and malaria was not systematically assessed in this study [32]. The fact that there was no difference at baseline between identified additional parasitic or filarial infections between the groups suggests that they did not differ in rates of non-*Loa* coinfection in the present study.

This study has exciting implications regarding the importance of eosinophils in mediating some of the symptoms associated with loiasis. Additional studies are under way examining the alterations in eosinophil granule proteins and cytokines following treatment to better understand the role played by eosinophils in posttreatment reactions. Indeed, an interventional study targeting the IL-5 axis is under way as a preventive strategy to mitigate some of the posttreatment reactions seen in loiasis.

Several important questions remain unanswered. The immune downregulation seen among END appears to be related to factors independent of microfilariae, but the underlying pathophysiology of these differences is not known. Nevertheless, the regulatory mechanisms responsible for the altered eosinophil counts and activation seen between the 2 groups and the contribution this makes to underlying symptomatology need to be better defined. This could help better understand the posttreatment symptoms seen in this important filarial infection and to identify methods to ameliorate these serious posttreatment adverse events.

Notes

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Author contributions. T. B. N., A. D. K., C. T.-W., M. L., and J. H. helped to codify the clinical information related to the presenting signs and symptoms of patients with loiasis and maintained the database of clinical information. J. H., M. M., and T. B. N. developed the eosinophil granule protein Luminex immunoassay. T. B. N. was responsible for the treatment and clinical care provided to all patients, and T. B. N., J. H., and A. D. K. provided some of the clinical care of the patients under protocol NCT00001230. J. H. performed all of the immunoassays with assistance from S. M. and J. H., and T. B. N. performed the data analysis. J. H. and T. B. N. wrote the manuscript, with all authors providing assistance with editing. T. B. N., as head of the Laboratory of Parasitic Diseases, oversaw all laboratory assays included in this study and was the primary person responsible for the intellectual development of the study. J. H., as corresponding author, had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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