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# Heightened Measures of Immune Complex and Complement Function and Immune Complex–Mediated Granulocyte Activation in Human Lymphatic Filariasis

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*Abstract.* The presence of circulating immune complexes (CICs) is a characteristic feature of human lymphatic filariasis. However, the role of CICs in modulating granulocyte function and complement functional activity in filarial infection is unknown. The levels of CICs in association with complement activation in clinically asymptomatic, filarial-infected patients (INF); filarial-infected patients with overt lymphatic pathologic changes (CPDT); and uninfected controls (EN) were examined. Significantly increased levels of CICs and enhanced functional efficiency of the classical and mannose-binding lectin pathways of the complement system was observed in INF compared with CPDT and EN. Polyethylene glycol–precipitated CICs from INF and CPDT induced significantly increased granulocyte activation compared with those from EN, determined by the increased production of neutrophil granular proteins and a variety of pro-inflammatory cytokines. Thus, CIC-mediated enhanced granulocyte activation and modulation of complement function are important features of filarial infection and disease.

## INTRODUCTION

Lymphatic filariasis (LF), a global disease that affects more than 129 million persons worldwide, is caused by the nematodes *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. The major pathologic consequences of chronic filarial infection are hydrocele, lymphedema, and elephantiasis.[1] Development of filarial pathologic changes is thought to be dependent on extrinsic factors (total parasite burden, intensity of transmission by infective stage larvae, and secondary bacterial infections) and intrinsic factors (host immune responses).[2,3]

Immune complexes (ICs) are heterogeneous high-molecular-weight aggregates composed of antigens, immunoglobulins, and complement components.[4] Accumulation of ICs leads to a broad spectrum of proinflammatory effects, including activation of the complement cascade and induction of cytokine secretion. These complexes may also be deposited in tissues and vessel walls, leading to inflammation, tissue damage and, ultimately, disease manifestations.[5] Immune complexes also affect disease progression and outcome in various disorders through the induction of pro-inflammatory or anti-inflammatory cytokines.[6–8] These complexes are potent activators of the complement system. The physiologic role of complement in the biology of circulating immune complexes (CICs) includes solubilization of ICs, prevention of immune precipitation, and the clearance of ICs from circulation through erythrocyte complement receptor 1 (CR1).[9]

Parasitic infections have repeatedly been associated with high levels of CICs and direct tissue-damaging effects of these complexes in mediating immune complex renal disease.[10] In chronic parasitic infections such as LF, alteration of parasite cell-surface molecules or immunogenic excretory/secretory antigens of the parasite can lead to formation of antigen-specific antibodies that can result, in turn, in the formation of IC. Several studies have reported the existence of high levels of CIC in filarial infections; some have explored the utility of IC for the diagnosis of infection with *W. bancrofti*.[11] Levels of IC have also been shown to fluctuate after antifilarial therapy.[12] Other studies have shown CIC levels to be associated with fever and adenolymphangitis in patients with Bancroftian filariasis.[13] Similarly, antigen-specific IC levels were significantly increased in 90% of persons with LF and overt disease manifestations.[11] Another study associated high CIC levels with low levels of C3, suggesting the use of complement by IC[14] in LF. Significant use of complement through the alternative pathway has also been observed with intrauterine eggs of many filarial species, and with microfilaria of *Brugia pahangi*, *B. malayi*, and *Litomosoides carinii*.[15] In addition, microfilaria can bind and inactivate complement and inhibit complement-mediated granulocyte chemotaxis.[16]

Despite these previous studies, there is a paucity of information on the interaction of CIC with the complement system. Moreover, the role played by IC in the modulation of innate immune responses observed in LF has also not been well studied. We hypothesized that the interaction of CIC/complement with the host innate immune system would be a major contributing factor in the development of lymphatic pathologic changes and/or host resistance. Thus, the present study assessed the role of CIC in LF by examining levels of CIC and the status of complement activation in each of the major pathways (classical, alternative, and mannose-binding lectin [MBL]) of complement activation. Once identified, the function of these ICs in mediating release of neutrophil granular proteins and cytokines/chemokines was assessed in the appropriate patient groups.

## MATERIALS AND METHODS

**Patient samples.** All samples were obtained as part of a study reviewed and approved by the Institutional Review Boards of the National Institute of Allergy and Infectious Diseases (Bethesda, MD) and the Tuberculosis Research Center

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(Chennai, India). Informed written consent was obtained from all participants and from respective parents in cases of pediatric participants.

This study was part of two larger studies (NCT00001230 and NCT00340691) performed in Chennai, India, an area to which *W. bancrofti* is endemic. One hundred twenty samples from persons in India were in this study. Uninfected controls (EN) were persons residing in filaria-endemic areas of southern India who were found to be free of *W. bancrofti* infection by negative test results for circulating filarial antigen (cAg) levels (by a qualitative immunochromatographic test; Binax, Cranfield, United Kingdom and a more quantitative enzyme-linked immunosorbent assay [ELISA]; Trop Bio Pty. Ltd., Townsville, Queensland, Australia), a negative test result for *B. malayi* antigen-specific IgG4, and no history of or clinical signs of LF. Clinically asymptomatic (subclinical) but filaria-infected persons (INF) were positive for cAg and filaria-specific IgG4. Persons with chronic pathologic changes who exhibited various degrees of lymphedema (stage II to frank elephantiasis) (CPDT) were negative for cAg but positive for *B. malayi*-specific IgG and IgG4, and all had been treated with diethylcarbamazine. The study populations are described in Table 1.

**Sample collection.** Plasma obtained during isolation of peripheral blood mononuclear cells was immediately aliquoted. Each aliquot of 500 µL was stored at –80°C and used only once after thawing.

**Levels of circulating immune complexes.** Circulating immune complexes were assayed by using the polyethylene glycol (PEG) precipitation method and an enzyme immunoassay (EIA) (Quidel Corp., San Diego, CA). For the PEG precipitation method,[17] 50 µL of plasma was incubated with an equal volume of 5% PEG-6000 overnight at 4°C. The mixture was then centrifuged at 2,000 rpm for 30 minutes at 4°C. The precipitate was washed twice in phosphate-buffered saline (PBS) and then dissolved in 500 µL of PBS, pH 7.4, for 30 minutes at room temperature. After incubation, the absorbance was read at 280 nm by using a spectrophotometer. Levels of CICs were determined from a standard curve plotted by using aggregated human gamma globulins as a standard. For cell culture assays, after washing with PBS, the IC precipitate was subsequently diluted to the initial plasma volume in PBS. For the EIA, plasma samples were added to the C1q-coated assay wells. Bound CIC was detected using goat anti-human Ig–horseradish peroxidase. The quantity of CIC in the sample (µg Eq/mL) was determined after the addition of substrate by comparison to a standard curve.

**Measurement of plasma complement.** Plasma levels of C3a, C4a, and C5a were measured by using commercially available ELISA kits (BD OptEIA™; Becton Dickinson Biosciences, Sparks, MD), and the levels of Bb, terminal complement complex (SC5b-9), and total hemolytic complement (CH50) were measured by using an EIA (Quidel Corp.), according to the manufacturer's guidelines.

The efficiency of complement activation was analyzed by using a complement system screen kit (Wieslab™; Euro-Diagnostica, Malmö, Sweden). The assay combines principles of the hemolytic assay for complement activation and use of labeled antibodies specific for neoantigen produced as a result of complement activation. The amount of neoantigen generated was proportional to the functional activity of complement pathways. Briefly, microtiter wells were coated with specific activators of the classical, MBL, or alternative pathways. Plasma was diluted in diluent containing specific blocker to ensure that only the complement was activated by the specific coating. The amount of C5b-9 was detected by using neoantigen-specific alkaline phosphatase–labeled antibody and substrate solution. The amount of complement activation correlates with the color intensity and was measured in terms of absorbance.

***In vitro* culture.** All cytokine-producing responder cells (granulocytes) used to study the effect of immune complex admixture were obtained from nine healthy volunteers. Characteristics of these individuals are shown in Table 2. Twenty milliliters of blood was obtained from each person. Granulocytes from each healthy volunteer were stimulated with ICs from one EN, one CPDT, and one INF. Granulocytes were isolated by using the Ficol Hypaque sedimentation method and dextran sedimentation.[18] Cells were cultured with RPMI 1640 medium in the presence of the PEG precipitates (10% volume/volume) isolated from EN, INF, and CPDT in 24-well tissue culture plates (Corning, Corning, NY) by using $5 \times 10^6$ granulocytes/well. The ICs from the three study groups were chosen randomly from the above mentioned 40 persons from each group (EN: 0.9–7.1 µg/mL, INF: 4.1–9.2 µg/mL, and CPDT: 1.6–4.3 µg/mL). The PEG precipitates isolated for cell culture assays were prepared fresh and used immediately without freezing or thawing. After six hours, culture supernatants were obtained and analyzed for granular proteins and cytokines/chemokines.

**Enzyme-linked immunosorbent assay.** Levels of cytokines and chemokines in the culture supernatants were measured by using the Bioplex Multiplex Cytokine Assay System (Bio-Rad Laboratories, Hercules, CA). Cytokines analyzed were granulocyte–macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ,) tumor necrosis factor-α (TNF-α), IL-12, IL-6, IL-1β, IL-2, IL-4, IL-10, IL-13, and IL-17. Chemokines analyzed were IL-8, macrophage inflammatory

TABLE 1
Demographics of the study groups*

| Characteristic | CPDT (n = 40)† | INF (n = 40) | EN (n = 40) |
|---|---|---|---|
| Median age, years (range) | 39.5 (15–66) | 41.5 (16–68) | 25 (20–49) |
| Sex, M:F | 21:19 | 20:20 | 31:9 |
| ICT card test (no. positive/no. negative) | 0/40 | 40/0 | 0/40 |
| Trop Bio ELISA (no. positive/no. negative) | 0/40 | 40/0 | 0/40 |
| Median C antigen levels, units (range) | < 32 | 819 (132.9–32,768) | < 32 |
| IgG levels (µg/mL), GM (range) | 180.78 (8.9–1,729.2) | 269 (8.03–2,174.8) | 6.7 (1.1–61.3) |
| IgG4 levels (ng/mL), GM (range) | 141.4 (10.1–1,317.4) | 299 (5.2–3,134.6) | 49 (1.15–61.3) |

*CPDT = filarial-infected patients with overt lymphatic pathologic changes; INF = filarial-infected patients; EN = uninfected controls; ICT = immunochromatographic test; ELISA = enzyme-linked immunosorbent assay; GM = geometric mean.
†All previously treated with diethylcarbamazine.

TABLE 2
Demographics of the uninfected controls (healthy volunteers) used for the *in vitro* assay*

| Characteristic | EN (n = 9) |
|---|---|
| Median age, years (range) | 26 (24–30) |
| Sex, M:F | 5:4 |
| ICT card test (no. positive/no. negative) | 0/9 |
| Trop Bio ELISA (no. positive/no. negative) | 0/9 |
| Median C antigen levels, units | < 32 |
| IgG levels (μg/mL) GM (range) | 3.4 (1.15–13.6) |
| IgG4 levels (ng/mL) GM (range) | 15.4 (0.4–65.4) |

* EN = uninfected controls; ICT = immunochromatographic test, ELISA = enzyme-linked immunosorbent assay; GM = geometric mean.

protein-1α (MIP-1α), MIP-1β, monocyte chemoattractant protein (MCP), platelet-derived growth factor (PGDF), granulocyte colony-stimulating factor (G-CSF), and eotaxin. Levels of chemokine (C-X-C motif) ligand 1/cotton rat growth-regulated protein-α (CXCL1/GROα) were estimated by using an ELISA Kit (R&D Systems, Minneapolis, MN). Levels of myeloperoxidase, lactoferrin, and elastase were assessed by using commercially available ELISA Kits (Hycult Biotech, Uden, The Netherlands).

**Statistical analysis.** The geometric mean (GM) was used as the measure of central tendency. Comparisons were made by using the non-parametric Mann-Whitney U test, and all data were corrected for multiple comparisons by using the Bonferroni correction. *P* values < 0.05 indicated statistical significance. All statistics were performed by using GraphPad Prism version for Windows (GraphPad Software Inc., La Jolla, CA).

## RESULTS

**Levels of CIC.** To study the role of CICs in LF, we measured the CIC levels in plasma of CPDT, INF, and EN by using an EIA (CIC-C1q ELISA) and a PEG precipitation method (Figure 1). Irrespective of the method used to measure CICs, INF had significantly ($P < 0.001$) higher levels of CIC than either CPDT or EN. The INF had a GM of 6.5 μg/mL (by EIA) or 6.2 μg/mL (by PEG precipitation) in comparison with GMs, which ranged from 2.4 to 2.8 μg/mL for CPDT and from 2.2 to 2.4 μg/mL for EN.

**Levels of classical pathway–specific complement activation fragments.** To study the status of the complement system in LF, we measured CH50 (a functional measurement of the entire cascade) and individual components (C3a, C4a, C5a, Bb, and SCb-9) in all study participants (Figure 2). Levels of CH50 were significantly higher in INF (GM = 183.8 UEq/mL) than in CPDT (GM = 84.39 UEq/mL) or EN (GM = 106.8 UEq/mL) ($P < 0.009$ for INF versus EN and $P < 0.0001$ for INF versus CPDT), indicating heightened potential for activation in INF (Figure 2A).

Because the functional complement components were higher in patently infected patients, we measured individual activation fragments of the complement system. As shown in Figure 2B and Figure 2C, levels of C3a and terminal fragment SC5b-9 were also significantly higher in INF (C3a, GM = 4,744.28 ng/mL; SC5b-9, GM = 291.16 ng/mL) than in CPDT (C3a, GM = 2,422.86 ng/mL; SC5b-9, GM = 194.27 ng/mL) or EN (C3a, GM = 2,310.22 ng/mL; SC5b-9, GM = 164.28 ng/mL) ($P < 0.0001$ for both comparisons). No significant differences were observed between CPDT and EN.

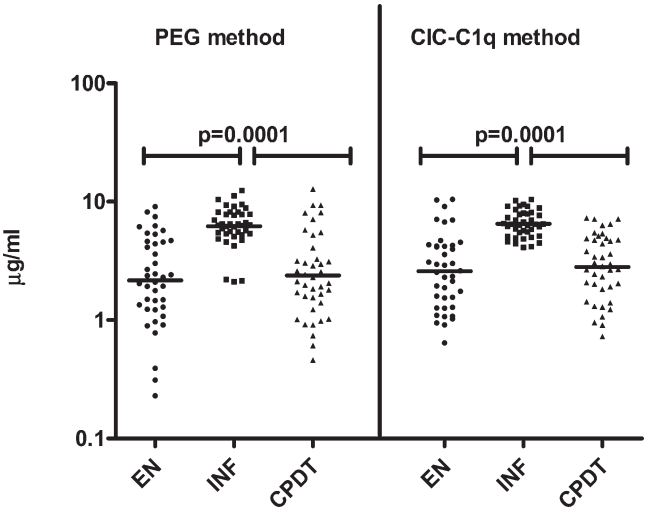


FIGURE 1. Increased levels of circulating immune complexes (CICs) in clinically asymptomatic, filarial-infected patients (INF) compared with filarial-infected patients with overt lymphatic pathologic changes (CPDT) and uninfected controls (EN). CIC levels (μg/mL) were assayed by using CIC-C1q enzyme-linked immunosorbent assay and polyethylene glycol (PEG) methods. Statistical significance was assessed by using the Mann-Whitney U test and *post hoc* Bonferroni methods. The number of persons in each group was 40.

As shown in Figure 2D, C4a levels were higher in INF (GM = 7,949.1 ng/mL) than in the other two study groups ($P < 0.0001$ for both comparisons). In addition, C4a levels were also higher in CPDT (GM = 4,389.73 ng/mL) than in EN (GM = 1,720.79 ng/mL) ($P < 0.0001$). Interestingly, C5a levels (Figure 2E) were higher in CPDT (GM = 608.5 ng/mL) than in the other two study groups ($P = 0.003$ for CPDT versus INF and $P = 0.004$ for CPDT versus EN). Levels of C5a were also higher in INF (GM = 452.1 ng/mL) than in EN (GM = 292.9 ng/mL) ($P = 0.03$). Similarly, levels of Bb (Figure 2F) were higher in CPDT (GM = 489.46 ng/mL) than in INF (GM = 345.27 ng/mL; $P = 0.0001$) or in EN (GM = 386.57 ng/mL; $P = 0007$).

**Activation of classical and MBL pathways in patent filarial infections.** Because total hemolytic potential and multiple complement fragments were increased in INF (some increases were also seen in CPDT), we assessed activation states of each of the major pathways (classical, alternative, and MBL). As shown in Figure 3, activities in the classical and MBL activation pathways were significantly ($P < 0.0001$, for each comparison) increased in INF (GM % activity: classical pathway = 42%; MBL = 76.3%) than in CPDT (classical pathway = 25.4%; MBL = 41.5%) or EN (classical pathway = 24.4%; MBL = 32.7%). No significant differences were observed among the three groups in the alternative activation pathway for complement.

**Effect of PEG-precipitated IC from patients with filarial infection function on release of granular proteins from normal granulocytes.** To determine the effect of CIC on normal granulocyte function, we stimulated granulocytes from normal healthy volunteers with IC isolated from INF, CPDT, and EN and measured levels of myeloperoxidase, lactoferrin, and elastase. Release of these granular proteins is an important indicator of neutrophil functional competence. As shown in Figure 4, IC from CPDT (GM = 64.39 ng/mL) induced higher levels of myeloperoxidase compared with IC from EN (GM = 30.69 ng/mL) and INF (GM = 49.36 ng/mL). The IC from INF

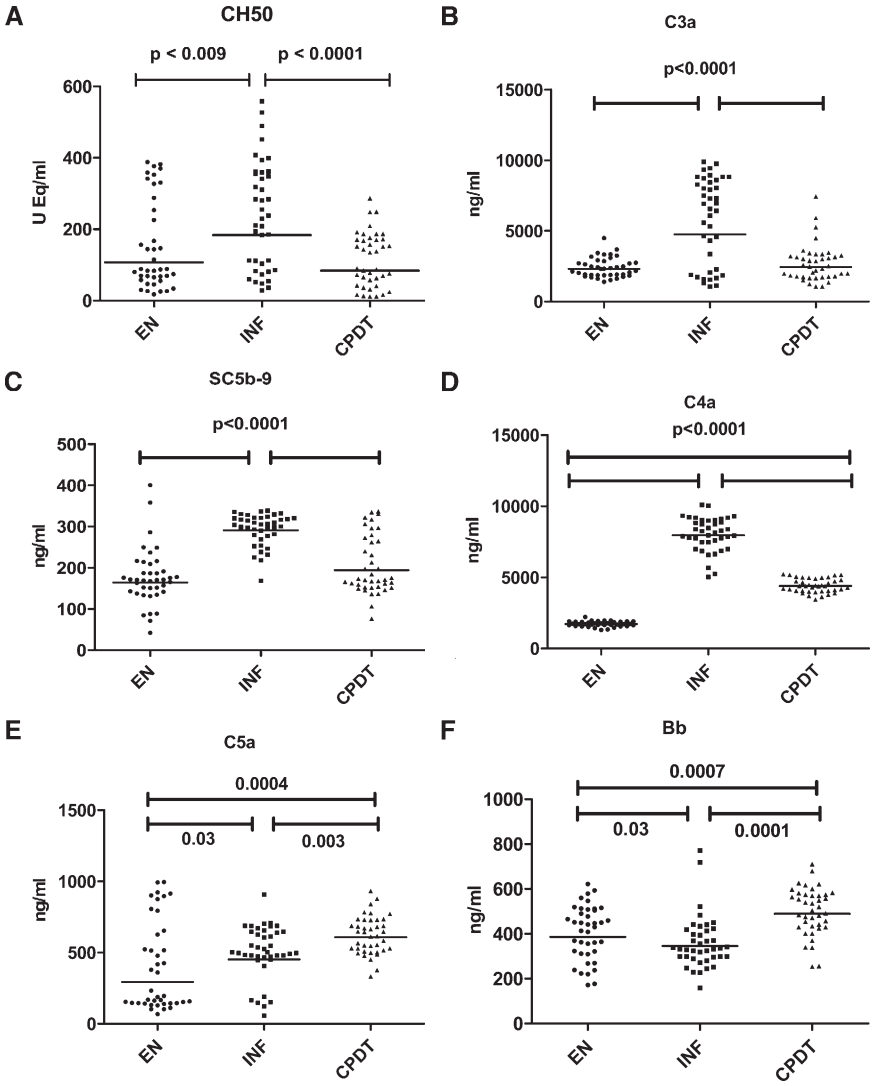


FIGURE 2. Increased complement activation fragments in patients with filariasis compared with filarial-infected patients with overt lymphatic pathologic changes (CPDT) and uninfected controls (EN). Levels of total hemolytic complement (CH50) (**A**) and complement activation fragments; C3a (**B**), SC5b-9 (**C**), C4a (**D**), C5a (**E**), and Bb (**F**) were estimated by using an enzyme-linked immunosorbent assay. Statistical significance was assessed by using the Mann-Whitney U test and *post hoc* Bonferroni methods. INF = clinically asymptomatic, filarial-infected patients. The number of persons in each group was 40.

(GM = 106.5 ng/mL) induced increased levels of lactoferrin than IC from EN (GM = 55.48 ng/mL) and CPDT (GM = 86.9 ng/mL). In contrast to myeloperoxidase and lactoferrin, IC from both filarial patient groups (INF, GM = 79.5 ng/mL and CPDT, GM = 83.7 ng/mL) similarly induced increased levels of elastase than IC from EN (GM = 48.16 ng/mL). Thus, although levels of IC were different in INF and CPDT, the functional activity of IC from the filarial patient groups was significantly more potent than that from uninfected persons.

**Effect of PEG-precipitated IC from patients with filariasis on production of pro-inflammatory cytokines.** To determine the effect of CICs on granulocyte cytokine production, we stimulated granulocytes from normal healthy volunteers with IC isolated from INF, CPDT, and EN individuals and measured the levels of cytokines (GM-CSF, IL-1β, IL-4, IL-6, TNF-α, IL-17, IFN-γ, IL-12, IL-2, IL-10, and IL-13). As shown in Figure 5, compared with EN, IC from INF and CPDT induced higher levels of IL-6 (GM for EN, 5.5 pg/mL; INF, 22.7 pg/mL; CPDT, 32.7 pg/mL) and IL-17 (GM for EN, 21.6 pg/mL; INF, 44.7 pg/mL; CPDT, 41.4 pg/mL). In addition, levels of IC-induced GM-CSF in INF (GM = 7.6 pg/mL) were higher than in EN (GM = 1.9 pg/mL) and CPDT (GM = 2.7 pg/mL). In contrast to pro-inflammatory cytokines, IL-4 was reduced when IC from INF (GM = 0.9 pg/mL) and CPDT (GM = 0.9 pg/mL) were incubated with normal granulocytes than with IC from EN (GM = 2.9 pg/mL). No significant differences were found among the three study groups for levels of IFN-γ, IL-1β, and TNF-α, and levels of IL-12, IL-2, IL-10, and IL-13 were not detectable in the supernatants.

**Effect of PEG-precipitated IC from patients with filariasis on PGDF, IL-8, and CXCL1/GROα.** To determine the effect of CIC on chemokine production by granulocytes, we stimulated granulocytes from normal healthy volunteers with IC isolated from INF, CPDT, and EN and measured levels of chemokines (IL-8, MIP-1α, MIP-1β, MCP, PGDF, G-CSF, eotaxin, and CXCL1/GROα). As shown in Figure 6, compared with EN and CPDT, IC from INF induced lower levels of GROα (GM for EN, 781.9 pg/mL; INF, 620.2 pg/mL; CPDT,

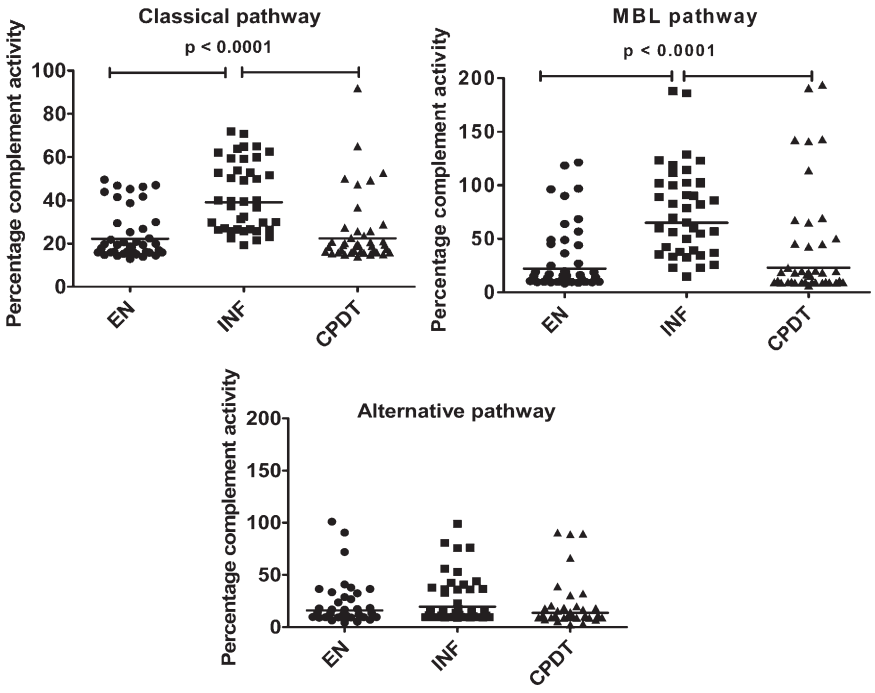


FIGURE 3. Enhanced classical and mannose-binding lectin (MBL) pathway activation of the complement system in clinically asymptomatic, filarial-infected patients (INF) compared with filarial-infected patients with overt lymphatic pathologic changes (CPDT) and uninfected controls (EN). Percentage activity of the three pathways of complement activation was assessed by using a complement functional screen kit. Statistical significance was assessed by using the Mann-Whitney U test and *post hoc* Bonferroni methods. The number of persons in each group was 40.

729.8 pg/mL) and IL-8 (GM for EN, 5,328 pg/mL; INF, 3,095 pg/mL; CPDT, 5,403 pg/mL). The IC from CPDT (GM = 5,254 pg/mL) induced higher levels of PDGF than IC from EN (GM = 3,031 pg/mL) and IC from INF (GM = 3,249 pg/mL).

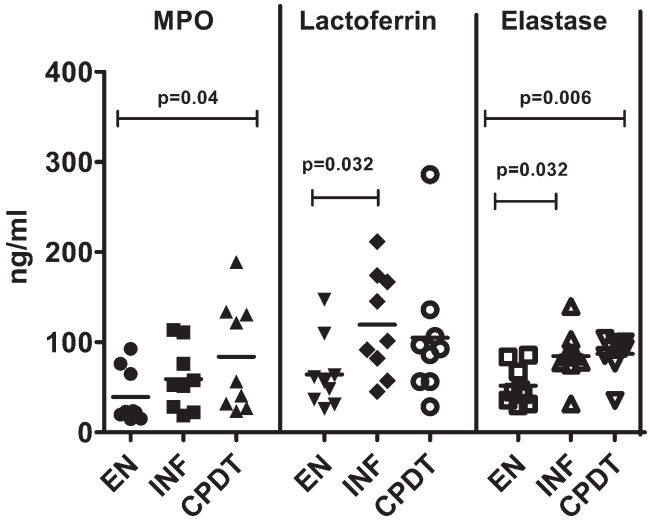


FIGURE 4. Release of granular proteins myeloperoxidase (MPO), lactoferrin, and elastase induced by polyethylene glycol–precipitated plasma immune complex from patients with filariasis. Granulocytes from normal healthy volunteers (n = 9) were stimulated with polyethylene glycol precipitates of plasma immune complex from filarial-infected patients (INF), filarial-infected patients with overt lymphatic pathologic changes (CPDT), and uninfected controls (EN) (n = 9 per group) for 6 hours, and cytokines were measured by using an enzyme-linked immunosorbent assay. *P* values were calculated by using the Mann-Whitney U test.

Levels of MIP-1α, MIP-1β, MCP, G-CSF, and eotaxin were not significantly different between the study groups.

## DISCUSSION

Several studies have examined the levels of ICs in various filarial infections. Persons with lymphatic filarial infections and overt clinical disease exhibited significantly increased levels of antigen-specific ICs in their circulation than microfilaria carriers.[11] Similarly, levels of antigen-specific ICs were higher in infected persons with dermal onchocercal lesions than in persons without such clinical changes.[19] It was previously reported that patients with LF had decreased levels of CIC compared with uninfected controls.[20] However, another study reported that equivalent levels were present in uninfected controls and persons with LF.[21] In the present study, clinically asymptomatic infected patients showed higher levels of CIC than patients with chronic lymphatic obstruction or uninfected controls. Given that INF have high levels of cAg and robust antibody production, this finding is not surprising. The relatively low CIC levels in patients with chronic lymphatic obstruction most likely reflect relatively reduced cAg levels in the circulation, which reflect lack of active disease. Because of widespread and chronic use of diethylcarbamazine in India, a drug that has both macrofilarcidal and microfilaricidal activities, relatively low levels of CIC in patients with chronic lymphatic obstruction may be related to the decrease often seen after treatment.[12]

Immune complexes are known to be a potent source of complement activation through the classical and alternative pathways. The MBL pathway is homologous to the classical pathway and the prototypical MBL ligand mannan can induce complement activation via the lectin pathway and the

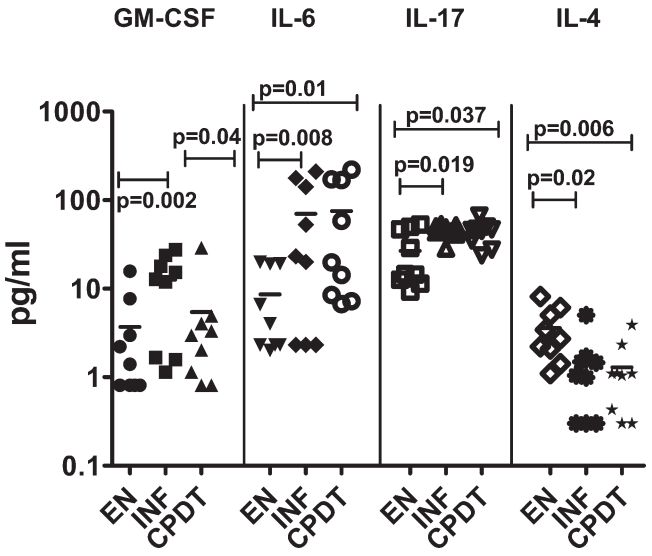


FIGURE 5. Release of pro-inflammatory cytokines by polyethylene glycol–precipitated plasma immune complex from patients with filariasis. Granulocytes from normal healthy volunteers (n = 9) were stimulated with polyethylene glycol precipitates of plasma immune complexes from filarial-infected patients (INF), filarial-infected patients with overt lymphatic pathologic changes (CPDT), and uninfected controls (EN) (n = 9 per group) for 6 hours, and cytokines were measured by using a multiplex enzyme-linked immunosorbent assay. *P* values were calculated by using the Mann-Whitney U test. GM-CSF; granulocyte–macrophage colony-stimulating factor; IL-6 = interleukin-6.

classical pathway. The presence of high levels of IC in INF explains the high levels of complement activation fragments, although other indicators of acute-phase reactivity was not examined in the present study. Nevertheless, a positive correlation was observed between activation of the complement

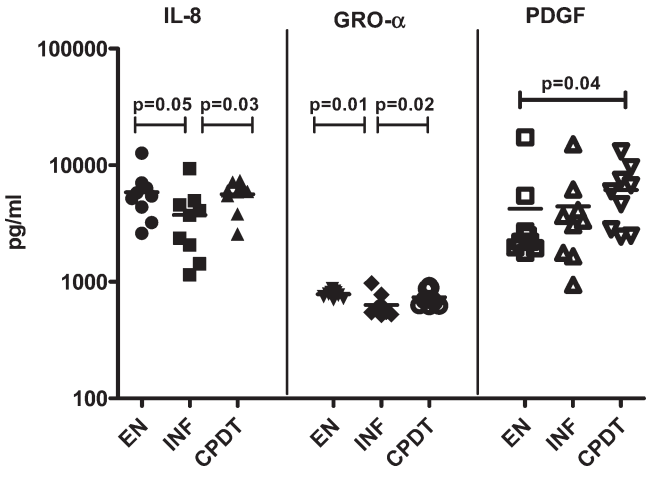


FIGURE 6. Modulation of release of interleuklin-8 (IL-8), chemokine (C-X-C motif) ligand 1, and platelet-derived growth factor (PDGF) by polyethylene glycol–precipitated plasma immune complex from patients with filariasis. Granulocytes from normal healthy volunteers (n = 9) were stimulated with polyethylene glycol precipitates of plasma immune complex from filarial-infected patients (INF), filarial-infected patients with overt lymphatic pathologic changes (CPDT), and uninfected controls (EN) (n = 9 per group) for 6 hours, and chemokines were measured by using a multiplex enzyme-linked immunosorbent assay. *P* values were calculated by using the Mann-Whitney U test. GRO-α = cotton rat growth-regulated protein-α.

system through the classical and MBL pathways and levels of IC. These data suggest that higher levels of CIC augment complement system activation in asymptomatic infected patients with filariasis, a response likely to reflect a host protective strategy because the MBL pathway has been associated with resistance to filarial infection.[22–24]

In terms of pathogenesis of lymphatic disease in LF, there is growing evidence to suggest that recurrent bacterial infections are an essential cofactor in development of lymphedema and its progression to elephantiasis.[3] The complement system is a fundamental element of the normal host defense against infection.[25] It is clear from our results that there is increased activation of the complement system in INF, which is likely through classical/MBL pathways, as evident from the complement functional screen test results and high levels of C3a, C4a, C5a, SC5b-9, and CH50. In the case of CPDT, the activation status of the complement system is comparable with that of EN (except for C5a and Bb activation fragments), as shown by levels of C3a, C4a, SC5b-9, CH50, and results of the complement system screen assay. This reduced activation of the complement system seen in CPDT might lead to an increase in secondary bacterial infections because effective antimicrobial activity is the main beneficial result of complement activation.

Nevertheless, high levels of C5a and Bb were observed in CPDT, and it is intriguing as to why there are increased levels of C5a and Bb in CPDT, despite reduced complement system activation, as assessed by the functional screen test. The difference could be that the complement functional screen test is also an indication of the functional integrity of the entire complement pathway and therefore a reflection of the function of all individual complement fragments, whereas levels of C5a and Bb fragments indicate the status of only two activation fragments. The increased vascular permeability observed in patients with chronic pathologic changes might lead to rapid deposition of ICs in vessel walls,[26] which might lead to IC-mediated tissue damage, thereby worsening pathologic changes seen in CPDT.

For infections with *Onchocerca volvulus* (a different but pylogenetically related filarial parasite), the inflammatory response has been shown to be influenced by antibody and complement interaction with the organism.[27] In addition, onchocerciasis is characterized by IC that contains IgM, which has been shown to exert a regulatory role on T cell function.[28] Immune complexes are also known to inhibit T and B lymphocyte function and antigen presentation by macrophages. Because it has been shown that ICs have potent immunomodulatory effects,[29–31] the effect of CIC from INF (and CPDT) on release of cytokines, chemokines, and granular proteins was studied. Using PEG-precipitated IC from patients with LF (compared with those without LF), we found that CIC could induce GM-CSF, IL-6, and IL-17 and inhibit the release of IL-4. We previously showed that induction of IL-17 is a prominent feature in patients with filaria-induced lymphedema[32] Similarly, IL-6 and GM-CSF are pro-inflammatory markers previously reported to be induced in filarial infections.[33,34] Thus, our results suggest that ICs might play an important role in pro-inflammatory cytokine production in filarial infections. Immune complex–mediated granulocyte immunomodulation also affects another cytokine, IL-4, which is known to be associated with resistance to filarial infection.[35]

In addition to cytokines, the effect of IC isolated from patients on chemokines was studied. Platelet-derived growth

factor, a regulator of fibrosis,[36] was found to be increased by ICs from CPDT, suggesting a role for these complexes in matrix turnover in these patients. In addition, chemotactic and angiogenic factors IL-8 and GRO-α were decreased in patients infected with filariasis without pathologic changes, indicating a possible role for IL-8 and GRO-α in development of filarial pathologic changes. Thus, ICs play an important immunomodulatory role in filarial infections, both in asymptomatic infections and perhaps in development of lymphatic pathologic changes. Interestingly, although levels of CIC were significantly higher in INF than in CPDT, the functional activity of CIC from both filaria-infected groups was not different in terms of its effect on granulocyte function, suggesting that the function of CIC in filarial infection is more a reflection of infection status (active or previous infection) than overt clinical pathologic changes.

Release of granular proteins from neutrophils during phagocytosis plays a role in microbicidal activity and tissue damage. Granule proteins also induce monocyte recruitment and enhance macrophage antimicrobial activity. Because monocyte extravasation and macrophage function depend on neutrophils and their secreted products[37] and monocyte dysfunction in filarial infection has been proposed as one mechanism underlying the diminished antigen-specific T cell response seen in patent LF,[38] we examined the effect of ICs on granular protein release. We found that ICs from patients with LF (INF and CPDT) increased release of granular proteins from normal granulocytes. In addition, IC-induced granular release might be beneficial to the host in combating secondary bacterial infections seen in these patients. The role of receptors (IgG/FcR, Toll-like receptors, and/or complement receptors) involved in IC-mediated granulocyte activation in filarial infection needs to be examined in future studies.

Our study highlights the role of CIC and the complement system in the disease pathogenesis of LF. High levels of circulating antigen, in combination with antigen-specific antibody, activate the complement system in asymptomatic persons, whereas the reduced status of complement activity in patients with chronic pathologic changes may aggravate disease morbidity. In addition, as demonstrated for loaisis, acquisition of complement regulators by the parasite can also serve to evade complement-mediated host resistance mechanisms.[39] Moreover, CIC from patients with filariasis can modulate release of granular proteins, proinflammatory cytokines, chemokines, and complement activation fragments. Although INF are clinically asymptomatic, it is clear from several studies that most of these persons have subclinical abnormalities. For example, lymphoscintigraphy studies have shown that asymptomatic infected persons have subclinical disease with considerable structural abnormalities and aberrant lymph flow.[40–42] In addition, subclinical lymphatic dysfunction was detectable in LF-infected children despite the absence of clinically evident lymphedema.[43] Therefore, CIC in LF constitutes a complex network modulating the levels of complement fragments, granular proteins, and cytokines/chemokines, which turn profoundly influences disease manifestations in this infection.

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