Expanded Numbers of Circulating Myeloid Dendritic Cells in Patent Human Filarial Infection Reflect Lower CCR1 Expression


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Expanded Numbers of Circulating Myeloid Dendritic Cells in Patent Human Filarial Infection Reflect Lower CCR1 Expression

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APC dysfunction has been postulated to mediate some of the parasite-specific T cell unresponsiveness seen in patent filarial infection. We have shown that live microfilariae of Brugia malayi induce caspase-dependent apoptosis in human monocyte-derived dendritic cells (DCs) in vitro. This study addresses whether apoptosis observed in vitro extends to patent filarial infections in humans and is reflected in the number of circulating myeloid DCs (mDCs; CD11c−CD123bh) in peripheral blood of infected microfilaremics. Utilizing flow cytometry to identify DC subpopulations (mDCs and plasmacytoid DCs [pDCs]) based on expression of CD11c and CD123, we found a significant increase in numbers of circulating mDCs (CD11c−CD123bh) in filaria-infected individuals compared with uninfected controls from the same filaria-endemic region of Mali. Total numbers of pDCs, monocytes, and lymphocytes did not differ between the two groups. To investigate potential causes of differences in mDC numbers between the two groups, we assessed chemokine receptor expression on mDCs. Our data indicate that filaria-infected individuals had a lower percentage of circulating CCR1+ mDCs and a higher percentage of circulating CCR5+ mDCs and pDCs. Finally, live microfilariae of B. malayi were able to downregulate cell-surface expression of CCR1 on monocyte-derived DCs and diminish their calcium flux in response to stimulation by a CCR1 ligand. These findings suggest that microfilaria are capable of altering mDC migration through downregulation of expression of some chemokine receptors and their signaling functions. These observations have major implications for regulation of immune responses to these long-lived parasites. The Journal of Immunology, 2010, 185: 6364–6372.

Antigen presenting cell dysfunction has been one mechanism used to explain the profound parasite-specific T cell hyporesponsiveness seen in chronic, patent lymphatic filariasis (1), although the mechanisms by which the filariae induce this have only been partially elucidated. Previously, we have shown that live microfilariae (mf) of Brugia malayi modulate dendritic cell (DC) function by two different mechanisms: 1) by altering TLR3 and TLR4 expression and function; and 2) by inducing apoptotic DC cell death (2, 3).

Two subsets of DCs have been identified in human blood (4) based on the pattern of expression of CD11c and CD123, with myeloid DCs (mDCs) being CD11c+CD123bh and plasmacytoid DCs (pDCs) being CD11c−CD123bh (5, 6). Although the function of these circulating DCs is not well understood, it is thought that these cells are in transit from either the bone marrow to peripheral tissues or from tissue to lymph nodes or spleen.

Following Ag/pathogen recognition, DCs mature and migrate from peripheral tissues to secondary lymphoid organs, where they present the Ag to lymphocytes (7, 8). This process of DC trafficking and migration is tightly regulated by chemokine receptor expression on these cells and their response to chemokine ligands (9). To this end, it has been shown that immature mDCs express functional CCR1 and CCR5, can respond to their ligands (MIP-1α and RANTES for both CCRs and MCP3, a chemokine that signals through CCR1 and CCR2), and migrate to sites of inflammation. During the process of maturation and in response to inflammatory stimuli, DCs downregulate their cell surface expression of these chemokine receptors and upregulate their expression of CCR7 (reviewed in Ref. 9).

Our present study addresses whether apoptosis observed in vitro is reflected in the number of circulating mDCs in the peripheral blood of microfilaria-positive, filaria-infected individuals (Fil*). By flow cytometry, we show that Fil* have a significantly higher number of circulating mDCs compared with their uninfected counterparts (Fil–). Furthermore, the number of other APCs (pDCs, monocytes [MΦ], and macrophages [MΦ]) was not different between the two groups. Filarial infection was associated with a lower percentage of CCR1-expressing mDCs that could be modeled in vitro by microfilaria–DC interactions. These findings collectively suggest that mf are capable of altering mDC migration through changes in chemokine receptor-mediated signaling, thereby altering DC homeostasis.
Materials and Methods

Study populations

The study village, N’Tessoni, located ∼385 km southeast of Bamako, is in a region of Mali endemic for the filarial parasites Wuchereria bancrofti and Mansonella perstans. The immunological study described in this paper was part of a larger study (http://www.ClinicalTrials.gov trial identification: NCT00341666) investigating clinical and immunological differences between Fil+ and Fil− subjects with respect to symptomatic malaria that was conducted in 2006 to 2007. Prior to the study, community permission for the study was obtained from village elders, and the study was approved by the ethical review committees (Institutional Review Boards) of the Faculty of Medicine, Pharmacy, and Dentistry at the University of Bamako (Bamako, Mali) and the National Institute of Allergy and Infectious Diseases (Bethesda, MD). Adults aged 18–65 y old were screened for this study, and written informed consent was obtained from all participants. Individuals who received treatment for filarial infections (albendazole or ivermectin) previously were excluded at screening.

Two study populations were identified for this immunological substudy: 1) individuals with confirmed active filarial infection as defined by the presence of circulating filarial Ag and/or mf of W. bancrofti and/or mf of M. perstans (Fil+; n = 9); and 2) individuals with no evidence of active W. bancrofti or M. perstans infection (circulating filarial Ag and mf negative, Fil−; n = 9). All microfilaremic individuals identified by our screening in the village were included in this study. Following our study, all residents of the village received ivermectin and albendazole treatment for lymphatic under the Malian Program for Elimination of Lymphatic Filariasis.

Study procedures

Each subject had a focused clinical evaluation for symptoms and signs of filarial infection and provided a blood sample that was transported to a laboratory in Bamako for processing. Hematologic analyses (including hemoglobin levels and total and differential WBC counts) were performed on all subjects. Additional laboratory analysis of the blood samples included blood smears for malaria parasites and mf ([W. bancrofti and M. perstans] and quantitation of circulating filarial Ag (TropBio, Townsville, Australia).

PBMC separation

PBMCs from the blood of Malian volunteers were isolated by Ficoll dextran density centrifugation as described previously (1, 10).

Flow cytometry

Sample staining. PBMCs were isolated from study volunteers, immediately fixed in 4% paraformaldehyde, and cryopreserved as previously described (11). Staining of cells with Abs was carried out according to standard protocols as follows. Cells were incubated with 10 μl human IgG (10 mg/ml; Sigma-Aldrich, St. Louis, MO) for 10 min at 4˚C to inhibit nonspecific binding through FcγRs. Cells were then incubated with marker-specific mAb conjugated with FITC, Alexa Fluor 700, APC, PE-Cy7, or PE at saturating concentrations for 30 min at 4˚C and washed twice with FACS medium. The mouse anti-human mAbs used in this study are listed in Supplemental Table I. mAbs specific for lineage markers Lin (Supplemental Table II), HLA-DR, CD11c, and CD123 were used in all samples to gate on mDCs and pDCs. Cells were also stained for surface expression of the chemokine receptors (CCR, CCR1, CCR5, CCR6, and CCR7). Monocyte cell populations were identified as cells that were positive for both CD14 and HLA-DR and macrophage populations as CD14+HLA-DR+CD68+.

Flow cytometric analysis. For flow cytometric analysis, 50,000 events were acquired per tube using a BD LSR II flow cytometer (BD Biosciences, San Jose, CA). Compensation was performed in every experiment using BD CompBeads (BD Biosciences) for single-color controls and unstained cells as negative controls. The flow cytometry setup for these experiments is shown in Supplemental Table II. Data were analyzed using FlowJo Software (Tree Star, Ashland, OR). The gating strategy used in this study is summarized in Fig. 1. Nonviable cells and granulocytes were excluded from our analysis on the basis of forward and side scatter. We calculated integrated geometric mean fluorescence intensity (GMFI) for CCR1 and CCR5 by multiplying the percentage of CCR1+ or CCR5+ cells in each population (mDCs or pDCs) by the corresponding GMFI of the chemokine receptors.

Normalization of the number of mDCs and pDCs in 1 ml blood

For analysis of DC and macrophage populations within the PBMC populations, we used gates that included both lymphoeytic and mononuclear cell populations identified by side and forward scatter (Fig. 1). The frequencies of DCs and MΦ, calculated from the percentage of target cells and the total number of mononuclear cells in the PBMCs analyzed, were normalized for the number of mononuclear cells per milliliter of whole blood from which the PBMCs were purified, using the total and differential counts of the blood (enumerated by a Coulter counter [Beckman Coulter, Fullerton, CA]). For example, the frequency of the monocyte population (HLA-DR+CD14+ cells) in a given individual per milliliter of blood was calculated by multiplying the percentage of this population by the total number of MΦ and lymphocytes per 1 ml blood.

Cytokine measurement

Levels of cytokines/chemokines in the serum of Fil+ and Fil− were measured by Searchlight proteome arrays (Aushon Biosystems, Billerica, MA). All samples were tested in duplicate, and the results are expressed as the mean of the replicates.

Calcium flux measurement

The response of cells to MCP3, MIP-1β, and RANTES was investigated by measuring intracellular calcium concentration changes using the FLIPR calcium 3 assay kit (PeproTech, Rocky Hill, NJ) according to the manufacturer’s protocol. Briefly, DCs and mf-exposed DCs were harvested.

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Table I. Clinical and parasitological characteristics of the study populations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fil−</th>
<th>Fil+</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Gender [n (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2 (22.3)</td>
<td>5 (55.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Female</td>
<td>7 (77.8)</td>
<td>4 (44.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Age (y), median (range)</td>
<td>38 (18–56)</td>
<td>54 (30–63)</td>
<td>0.019</td>
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<tr>
<td>Clinical signs of filariasis [n (% positive)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocele</td>
<td>0</td>
<td>1 (11.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Arm/leg edema</td>
<td>1 (11)</td>
<td>3 (33)</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphangitis</td>
<td>2 (22)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Elephantiasis</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>1 (11)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Laboratory parameters (mean ± SD)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>WBC count (per μl)</td>
<td>5.4 ± 0.4</td>
<td>6.2 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Eosinophil count (% of WBC)</td>
<td>11.4 ± 2.7</td>
<td>9.8 ± 1.4</td>
<td>NS</td>
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<tr>
<td>Microfilaria</td>
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<td></td>
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<tr>
<td>W. bancrofti n (% positive)</td>
<td>0</td>
<td>9 (100)</td>
<td>NS</td>
</tr>
<tr>
<td>mf per 60 μl [GM (range)]</td>
<td>–</td>
<td>252 (83–917)</td>
<td></td>
</tr>
<tr>
<td>M. perstans n (% positive)</td>
<td>0</td>
<td>8 (80)</td>
<td>NS</td>
</tr>
<tr>
<td>mf per 60 μl [GM (range)]</td>
<td>–</td>
<td>234 (33–950)</td>
<td></td>
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</table>

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washed, and plated on poly-L-lysine (Sigma-Aldrich) precoated 96-well black-wall clear plates at 1 × 10^5 cells/well in HBSS (Invitrogen, Carlsbad, CA). The plate was centrifuged at 2000 rpm for 5 min. The supernatant was discarded, and the calcium dye was diluted at 1:1 in HBSS and added to the cells (100 μl/well). The plate was incubated for 20 min at 37°C in 5% CO2. Cells were then centrifuged once for 5 min. Agonists were prepared in HBSS at several dilutions down to a final concentration of 100 nM. The plates were transferred directly to a FlexStation for measurement of fluorescence, and the average fluorescence values of the duplicates were used for each data set. Maximum – baseline fluorescence values, with negative control subtraction from each well, were calculated and graphed.

Preparation of mf
Live B. malayi mf were provided by Dr. Ray Kaplan (University of Georgia, Athens, GA) as described previously (12). Briefly, live mf were collected by peritoneal lavage from infected jirds and separated from peritoneal cells by Ficoll diatrizoate density centrifugation. The mf were then washed three times in RPMI 1640 with antibiotics and cultured overnight at 37°C in 5% CO2.

In vitro generation of DCs
CD14+ peripheral blood-derived Mφ were isolated from WBCs collected from healthy donors by counterflow centrifugal elutriation. Mφ were cryopreserved in liquid nitrogen at 5 × 10^7/vial and thawed for culture in six-well tissue culture plates (Costar, Cambridge, MA) at 2 to 3 × 10^6/ml in complete RPMI 1640 (BioWhittaker, Walkersville, MD) supplemented with 20 mM glutamine (BioWhittaker), 2% heat-inactivated human AB serum (Gemini Bioproducts, West Sacramento, CA), 100 IU/ml penicillin, and 100 μg/ml streptomycin (Biofluids, Rockville, MD). Recombinant human IL-4 and recombinant human GM-CSF (PeproTech) were added to the culture at 50 ng/ml on days 1, 4, and 6 of culture. Live mf were added on day 6 at a final concentration of 5 × 10^4/well (per 1 to 2 × 10^6 DCs). Cells were harvested at day 8 of culture after Versene/EDTA treatment (Biofluids) and washed twice with PBS (without Ca2+/Mg2+). Viability and cell counts were determined by trypsin blue exclusion and used for flow cytometric analysis or other functional studies. DCs harvested at day 8 were repeatedly shown to be CD1α+, HLA-DR+, CD86+, CD40+, CD3−, CD14−/hi, CD19−, and CD56− by flow cytometry (FACS Calibur, BD Biosciences).

Real-time RT-PCR
RNA (1 μg) from an infected individual (patient 6 from Table I) was used to generate cDNA and then assessed by standard multiplex TaqMan assays (Applied Biosystems, Foster City, CA). This individual was the only subject with sufficient RNA yields to perform this analysis on both pre- and posttreatment samples.

For RT-PCR, random hexamers were used to prime RNA samples for reverse transcription using Multiscribe reverse transcriptase (Applied Biosystems), after which PCR products for HSPA4, HSPA8, HSPCA, and HSPCB, as well as endogenous 18s rRNA controls, were assessed in triplicate wells using TaqMan assay reagents developed by the manufacturer (Applied Biosystems). These include PCR forward and reverse primers and a FAM dye-labeled TaqMan MGB (minor groove binder) probe, which crosses intron/exon boundaries to amplify only mRNA. For endogenous control, a set of primer and a VIC-labeled MGB 18S probe was used. Real-time quantitative RT-PCR was performed on an ABI 7700 sequence detection system (Applied Biosystems).

Statistical analysis
The nonparametric Mann–Whitney U test and the nonparametric Wilcoxon signed-rank test were used as indicated in each figure legend. Correlations were assessed using the Spearman rank correlation test. Statistical analyses were performed with StatView 5 (SAS Institute, Cary, NC) or GraphPad Prism Version 5.0 (GraphPad, San Diego, CA). In all comparisons, p values <0.05 were considered statistically significant.

**FIGURE 1.** Subtyping of mDCs and pDCs. mDC and pDC subpopulations were evaluated in fixed PBMC from Fil+ and Fil− individuals. Gated PBMCs (A) negative for expression of Lin markers and positive for expression of HLA-DR (B) were stained for expression of CD11c and CD123. HLA-DR−/Lin− populations that are CD11c+ and CD123lo are considered to be mDCs; those that are CD11c+ and CD123hi are considered to be pDCs (C). The monocyte population was identified based on gated PBMC (A) that were positive for expression of CD14 and HLA-DR (D) and the macrophage population based on the HLA-DR+/CD14− cells (D) that were positive for expression of CD68 (E).
Results

Study populations

Fil+ and Fil− residents of a single village were randomly selected after a screening visit during which blood samples were collected. Based on predefined selection criteria (see Materials and Methods), we identified nine Fil+ and nine Fil− individuals for this study. The two groups (Fil+ and Fil−) did not differ significantly in gender composition or clinical signs related to their filarial infections (Table I). The Fil+ group had a significantly higher infection rate (9/9; 100%) compared with the Fil− group (5/9; 56%) (Fig. 1). The two groups (Fil+ and Fil−) did not differ significantly in median age (54 y; range 30–63) than the Fil− group (38 y; range 18–56; p = 0.019), not unexpected in this setting, as filarial infections increase in prevalence with age in all endemic regions. WBC counts and eosinophil counts were also statistically equivalent in the two groups. All of the Fil+ individuals had circulating mDCs (Lin− DR+/CD14+ (DRA)+ CD123hi) based on established criteria (see Fig. 1 for the gating strategy), MΦ, and pDCs (Lin− DR+CD11c+CD68+) between the two groups (Fil+ and Fil−). Based on these analyses, we demonstrated a significant increase in the number of mDCs in Fil+ compared with Fil− individuals (Fig. 2A), with the GM for Fil+ being 87,794 cells/ml compared with 47,000 cells/ml for Fil− (p = 0.002).

Fil+ individuals had a higher number of circulating mDCs compared with Fil− individuals

Previously, we have shown that live mf of B. malayi induce apoptotic cell death in human mDCs (2). To assess whether our in vitro findings were reflected by alterations in circulating mDC numbers in vivo, PBMCs were immediately fixed and cryopreserved. PBMCs from the two groups (Fil+ and Fil−) were then examined for number of circulating mDCs (Lin− DR−CD11c−CD123hi), pDCs (Lin− DR−CD11c+CD123hi) based on established criteria (see Fig. 1 for the gating strategy), MΦ, and pDCs. Based on these analyses, we demonstrated a significant increase in the number of mDCs in Fil+ compared with Fil− individuals (Fig. 2A), with the GM for Fil+ being 87,794 cells/ml compared with 47,000 cells/ml for Fil− (p = 0.002).

Although slightly increased in the Fil+ group compared with Fil−, the number of pDCs did not differ significantly (Fig. 2B). In contrast, there was no difference in the number of circulating MΦ (HLA− DR+/CD14+) or MΦ (HLA− DR+/CD14+/CD68+) between the two groups (Fig. 2C, 2D). There was no relationship between the number of circulating mf and the number of circulating mDCs (p = 0.538; r² = 0.066) or pDCs (p = 0.12, r² = 0.352) in Fil+ individuals (data not shown).

Fil+ individuals have a lower percentage of circulating CCR1+ mDCs and a higher percentage of circulating CCR5+ mDCs and pDCs compared with Fil− individuals

To address whether the increased number of circulating mDCs observed in Fil+ is due to changes in subpopulations of DCs expressing particular CCRs known to mediate transendothelial migration in DCs, we assessed the expression of CCR1, CCR5, CCR6, and CCR7 on both mDCs and pDCs. Although there were no differences in the percentages of CCR6 and CCR7 cells in both mDC and pDC populations between the two groups (Figs. 3C, 3D, 4C, 4D), there was a significant decrease in the percentage of circulating CCR1+ mDCs in Fil+ compared with Fil− individuals (p = 0.01) (Fig. 3A). The percentage of CCR1+ pDCs was also slightly (but not statistically significant) lower in Fil+ individuals (GM 24.8 in Fil− versus 14.5 in Fil+) (Fig. 4A). In contrast, the percentages of circulating CCR5+ mDCs (p = 0.007) and pDCs (p = 0.01) were significantly higher in Fil+ compared with Fil− individuals (Figs. 3B, 4B). Furthermore, the per-cell surface expression of CCR1 as measured by iGMFI was also significantly lower on mDCs (p = 0.048) (Fig. 3E) but not on pDCs (Fig. 4E), and the per-cell surface expression of CCR5 was significantly higher in both mDCs and pDCs in the Fil+ as compared with the Fil− group (Figs. 3F, 4F).

We next assessed whether the difference in the percentage of CCR1+ or CCR5+ mDCs between Fil+ and Fil− were related to the
serum levels of their ligands (chemokines). We did not detect any MCP3 (CCR1 ligand) and very low levels of MCP2 (CCR1, CCR2, and CCR5 ligand) in the sera of both Fil+ and Fil individuals (GM 0.9 pg/ml). In addition, whereas MIP-1b (a CCR5-specific ligand) was detected in both groups, there were no differences in the levels of these chemokines between the groups. Furthermore, there were no differences between the groups in serum levels of eotaxin, MIP-3a, MIP-3b, IL-8, RANTES, or CCL16 (Fig. 5A, 5B).

Live mf of B. malayi downregulate expression of CCR1 on monocyte-derived DCs in vitro

Given that Fil+ individuals had a significant decrease in not only the percentage of circulating CCR1+ mDCs but also in the per-cell expression level of CCR1, we assessed the role of mf in directly mediating the decreased expression level of CCR1 using an in vitro model of mf–DC interaction. Ags and mf of B. malayi have been used extensively in place of W. bancrofti for in vitro studies of immune responses to filariae because propagation and maintenance of W. bancrofti is not feasible in nonhuman animals. In addition, the genetic makeup in the most genes products that have been identified to date for W. bancrofti and B. malayi are 90% homologous at the protein level.

As shown in Fig. 6, DCs exposed to live mf for 48 h showed diminished cell-surface expression of CCR1 (p = 0.03) compared with the mf-unexposed cells, whereas there was no difference in CCR5 expression. Thus, our in vitro data suggest that live mf can directly regulate the expression of CCR1 but not CCR5, CCR6, or CCR7 (data not shown) on mDCs.

Exposure to mf reduces CCR1 ligand-driven calcium flux in mDCs

To assess whether the diminished expression of CCR1 on DCs is reflected in diminished signaling through CCR1, we measured calcium flux in these cells in response to MCP3, a ligand that binds CCR1 (but not CCR5). We found that mf-exposed DCs exhibited significantly diminished calcium flux in response to MCP3 at 100 nM (DC/mf: maximum 2 baseline [RFU]; range 0–19) (Fig. 7A, 7B) as well as at 20 nM (data not shown) compared with the unexposed cells (DCs: maximum 2 baseline [RFU]; range 3–63). Furthermore, whereas there was a minimal increase in the intracellular calcium concentration of DCs in response to MIP-1b (a ligand that binds and signals through CCR5 but not CCR1), exposure to mf did not alter this response in 8 out of 10 donors (Fig. 7B). Interestingly, mf-exposed DCs showed a significant decrease in calcium flux in response to RANTES, a ligand that works through both CCR1 and CCR5, at 100 nM (DC/mf: maximum – baseline [RFU]; range 2–22) (Fig. 7B) and at 1 nM (data not shown), compared with unexposed DCs (DCs: maximum – baseline [RFU]; range 5–123), suggesting that an alteration in expression of CCR1 (and not CCR5) on DCs by mf resulted in a diminished ability of these cells to flux calcium in

FIGURE 3. Filaria-infected individuals have a lower percentage of circulating CCR1+ and a higher percentage of circulating CCR5+ mDCs compared with uninfected individuals. The percentage of CCR1+ (A), CCR5+ (B), CCR6+ (C), and CCR7+ (D) mDCs in filaria-infected (●, n = 6–7) and -uninfected (○, n = 9) individuals. iGMFI calculated for CCR1 (E) and CCR5 (F) show the per-cell expression of these receptors. The Mann–Whitney U test was used to test for statistical significance.
response to ligands (MCP3 and RANTES) that work through CCR1. Importantly, there was no statistically significant difference in calcium flux between mf-exposed DCs and unexposed DCs in their responses to ATP (Fig. 7B).

Discussion
The mechanisms of Ag-specific hyporesponsiveness observed in Fil+ individuals with mf have been the focus of intense investigation, and our previous studies have shed light on the role of APC dysfunction and mf in this phenomenon. We have shown that live mf of *B. malayi* can disrupt normal functioning of human monocyte-derived DCs in at least two ways: 1) by inducing a caspase-dependent apoptotic cell death (2); and 2) by negatively regulating their TLR expression and signaling (3), thereby inhibiting Ag-presenting function. In other human population studies, it has also been shown that when MΨ from mf individuals are stimulated in vitro with filarial Ag, there is diminished upregulation of TLR expression and diminished cytokine production in response to some TLR ligands (13). Our study was designed to test whether the apoptotic cell death observed in vitro (2) extend to Fil+ individuals with active filarial infection in vivo, resulting in a lower number of circulating mDCs. Unexpectedly, our data demonstrated a significant increase (rather than a decrease) in the number of circulating mDCs in microfilaremic individuals (Fig. 2A). Importantly, there was no difference between the groups in the number of other circulating APC including pDCs, MΦ, and MΨ (Fig. 2C, 2D). Although there was an increase in the frequency of circulating mDCs, there was no relationship between the level of microfilaraemia, the levels of parasite Ag (representing the adult worm load), and the number of these cells (data not shown), suggesting this increase in mDCs is not a direct consequence of the number of mf circulating in the infected individuals. Similarly, the frequency of CCR1+ DC subpopulations also
did not correlate with either microfilaremia or parasite Ag levels in the blood (data not shown).

Although our in vivo observations differ from what we would have predicted from our in vitro findings, there are several possible explanations for what we found in vivo. For example, the expansion in mDC population that we observed in microfilaremic individuals could reflect an increase in DC cell death in compartments such as the bone marrow, resulting in increased turnover of mDCs with more precursor cells moving through the circulation. Alternatively, an increase differentiation of MΨ to mDCs could be occurring in the periphery or a failure of DCs to migrate through the endothelial barrier based on trafficking defects such as altered chemokine/chemokine receptor homeostasis.

Chemokines and chemokine receptors play an important role in directing the migration of DCs (14–16). mDCs and pDCs isolated from human blood differ in their capacity to migrate to chemotactic stimuli (17), and the expression patterns of chemokine receptors on these cell populations play a key role in directing migration of DCs into specific tissues because tissue migration follows specific chemokine gradients (18–20). DCs perform widely different functions at different stages of maturation and traffic to specific microenvironments as dictated by the local chemokine milieu (19). Optimal responses to different sets of chemokines are generally regulated by changes in the patterns of CCR expression. For example, immature DCs expressing a specific pattern of CCR, that include CCR1, CCR5, and CCR6, are able to home to peripheral tissues, whereas mature DCs express CCR7, a key molecule for migration from the periphery to T cell areas of secondary lymphoid tissues (9).

To investigate the possibility that DC migration was impaired in Fil+ individuals, we studied the expression of CCR1, CCR5, CCR6, and CCR7 in both Fil+ and Fil− study populations. Our findings indicate that although there was a very small percentage of circulating mDCs expressing CCR1 in both groups, this sub-population of mDCs was significantly smaller in Fil+ individuals (Fig. 3A); expression of CCR1 per cell on mDCs as measured by iGMFI was also lower in Fil+ individuals (Fig. 3E). In contrast, the percentage of circulating CCR5+ mDCs and pDCs and per-cell expression of CCR5 was significantly higher in Fil+ individuals compared with Fil− individuals (Figs. 3B, 4B), with no significant

FIGURE 5. A and B, Serum levels of CCR1 or CCR5 ligands do not differ between the filaria-infected and -uninfected groups. Chemokine levels in sera of filarial-infected (●; n = 9) and -uninfected individuals (○; n = 9) were measured using proteome arrays. Each circle or box represents an individual. The Mann–Whitney U test was used to measure statistical significance.

FIGURE 6. Live B. malayi microfilariae downregulate cell-surface expression of CCR1 on monocyte-derived DCs. Cell surface expression of CCR1 (A) and CCR5 (B) on in vitro-generated DCs either unexposed (●) or after 48 h exposure to live microfilariae of B. malayi (●) in six donors. Each line represents an independent experiment (n = 6). The non-parametric Wilcoxon signed-rank test was used to measure significance.
differences seen in either CCR6 or CCR7. Interestingly, we did not see a corresponding difference in the serum levels of MCP2, CCL16 (ligands for CCR1), MIP-1β, RANTES, or eotaxin (ligands for CCR5) between the two groups (Fig. 5). Together with the observation that these changes were unrelated to concentration of circulating chemokines (Fig. 5) or expression of CCR6 and CCR7, these data would suggest that mDCs in Fil+ individuals have a reduced capacity to migrate into tissues. These findings also raise a question as to whether the expanded size of the mDC population reflects redistribution of these cells into the circulatory compartment or is a compensatory increase in the bone marrow in response to increased DC turnover. Redistribution of blood DCs has been observed in autoimmune diseases such as systemic lupus erythematosus, in which reduced numbers of blood DCs with corresponding accumulation of DCs in the inflammatory sites have been reported (21).

Although there is limited information on the role of chemokine receptor expression in DC homeostasis in humans, redistribution of other cell populations has been linked to expression of these receptors. For example, in Chagas disease and schistosomiasis, a change in the expression of chemokine receptors on PBMCs has been associated with progression of the disease (22, 23). In the case of severe chronic chagasic cardiomyopathy, a serious complication of Trypanosoma cruzi infection, diminished CCR5 and CXCR4 expression on PBMCs of infected individuals was associated with the onset of heart failure (22). In Schistosoma mansoni infections, unbalanced chemokine receptor activation with increased expression of CCR1 relative to CCR5 correlated with a severe form of the disease (23). Chemokine receptor expression can also be altered in viral infections such as chronic hepatitis C infection, in which reduced surface expression of CCR1 and CCR5 on peripheral blood CD8+ T lymphocytes results in lower CC chemokine responsiveness, which, in turn, may affect T cell recruitment to the infected liver, an important step in clearing the virus. The relationship between chemokine receptors and cell distribution has also been shown in the wart, hypogammaglobulinemia infection, and myelokathexis syndrome, a rare combined (B and T cell) immunodeficiency associated with abnormal retention of neutrophils in the bone marrow that is linked to CXCR4 dysfunction (25, 26).

Evidence for defects in the expression of certain chemokine receptors has also been reported in individuals with lymphatic filariasis. Among Fil+ individuals, those with chronic lymphatic pathology have an augmented expression of CCR9 and diminished expression of CXCR1 and CXCR3 compared with Fil− individuals (27). Furthermore, CCR9 expression on these cells correlated with the grade of lymphedema in these patients (27). Finally, in a murine model of onchocerciasis (river blindness), it has been
demonstrated that CXCR2 expression is critical for neutrophil infiltration to the cornea (28).

We wanted to determine the direct role of mf on the differences in chemokine receptor expression observed between infected and uninfected populations. Using an in vitro model, live mf significantly downregulated the cell-surface expression of CCR1 but did not alter the expression of CCR5 on DCs (Fig. 6), suggesting that mf may directly influence chemokine expression (and responsiveness) in mDCs in vivo. These data found functional correlates in the diminished signaling induced by CCR1 ligands.

Chemokine receptor-induced calcium signaling is not completely understood, although it has been used as a surrogate of CCR signaling. It has been shown that channel openers, as well as phospholipase C and protein kinase inhibitors, affect calcium flux in response to some of the CCR ligands (29). In this study, we measured the capacity of mf-exposed DCs to induce calcium flux in response to some CCR ligands. Our data indicate that mf-exposed DCs had a diminished ability to induce calcium flux in response to the CCR1 ligand MCP3 as well as to RANTES, a ligand that signals through both CCR1 and CCR5. Whereas there was a minimal increase in the intracellular calcium concentration of DCs in response to MIP-1β (a ligand that binds and signals through CCR5 but not CCR1), exposure to mf did not alter this response, suggesting that a specific alteration in expression of CCR1 (but not CCR5) on DCs by mf resulted in diminished ability of these cells to trigger a response through ligands such as MCP3 and RANTES that signal through this receptor. These data collectively suggest that mf downmodulate the cell-surface expression of CCR1 on DCs and thereby alter the levels of receptor-mediated signaling to CCR1 ligands.

In conclusion, our results suggest that Fil+ individuals have a significantly increased number of circulating mDCs compared with Fil− individuals from the same endemic region. Our studies also reveal an important finding that this mDC redistribution in Fil+ patients may be due to lower levels of CCR1 expression. Finally, we demonstrated the potential relevance of this mechanism in human infections by showing that live mf negatively regulate the expression and function of CCR1 on monocyte-derived DCs, which in turn may be one mechanism for the aberrant redistribution of this important cell population.

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Disclosures
The authors have no financial conflicts of interest.

References

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