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Chemokine Responses Are Increased in HIV-Infected Malawian Children With Invasive Pneumococcal Disease

Enitan D. Carrol, MBChB, MRCPCH, MD[*,†,‡,§], **Limangeni A. Mankhambo, MBBS**[*,†], **Paul Balmer, PhD**[‖], **Standwell Nkhoma, PhD**[*], **Daniel L. Banda, DBS**[*], **Malcolm Guiver, PhD**[‖], **Graham Jeffers, MSc**[‡], **Nick Makwana, MBChB, MRCPCH**[‡,§], **Elizabeth M. Molyneux, FRCPCH**[†], **Malcolm E. Molyneux, FRCP**[*], **Rosalind L. Smyth, MD, FRCPCH**[‡], **C. Anthony Hart, PhD, FRCPath**[§], and **the Invasive Pneumococcal Disease Study Group**[*]

[*]Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi

[†]Department of Paediatrics, College of Medicine, University of Malawi, Blantyre, Malawi

[‡]Division of Child Health, University of Liverpool, Royal Liverpool Children's Hospital, Liverpool, United Kingdom

[§]Division of Medical Microbiology, The University of Liverpool, Duncan Building, Liverpool, United Kingdom

[‖]Health Protection Agency, Manchester Medical Microbiology Partnership, Manchester Royal Infirmary, Manchester, United Kingdom.

Abstract

Background: Chemokines play an important role in the recruitment and regulation of leukocyte traffic during bacterial infection. The aims of this study were to investigate the chemokine response to invasive pneumococcal disease (IPD) and to examine the influence of HIV infection on the chemokine response, pneumococcal bacterial loads, and outcome.

Methods: We prospectively studied 95 children with IPD, and blood and cerebrospinal fluid (CSF) samples were taken at admission for the determination of chemokines, interferon-γ (IFNγ), and pneumococcal bacterial loads.

Results: Plasma CXCL8 and CCL2, CSF CXCL8 and CCL4, and IFNγ were significantly higher in HIV-infected children than in HIV-uninfected children. Blood and CSF pneumococcal bacterial loads correlated with plasma and CSF chemokines, respectively, and were higher in HIV-infected children compared with HIV-uninfected children. Among HIV-infected children, plasma concentrations of CXCL8 and CCL2 were significantly higher in nonsurvivors than in survivors, but CCL5 was significantly lower. HIV-infected and HIV-uninfected children with IPD had higher concentrations of chemokines (except CCL5) than acutely ill HIV-infected and HIV-uninfected children with no detectable bacterial infection. Male gender and low plasma CCL2 concentrations were shown to be independently associated with survival.

Conclusions: Chemokines, in particular CCL2, are associated with survival in IPD and correlate with pneumococcal bacterial loads, disease presentation, and outcome.

Correspondence: Enitan D. Carrol, MBChB, MRCPCH, MD, Malawi Liverpool Wellcome Research Programme, PO Box 30096, Chichiri, Blantyre 3, Malawi (edcarrol@liv.ac.uk)..

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Keywords

bacterial loads; chemokines; HIV; invasive pneumococcal disease

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Streptococcus pneumoniae (the pneumococcus) is estimated to cause approximately 1 million deaths per year in children in the developing world.1 The pneumococcus is the primary cause of pneumonia and meningitis in children in the developing world1 and is the leading cause of community-acquired bacteremia in children in sub-Saharan Africa.2 The burden of invasive pneumococcal disease (IPD) has increased in areas with a high prevalence of HIV infection.3 In South Africa, the disease incidence in children younger than 2 years of age has been shown to be 73 per 100,000 children per year in HIV-uninfected children compared with 3036 per 100,000 children per year in HIV-infected children.4

Chemokines (or chemoattractant cytokines) are subdivided into 4 distinct subfamilies (CC, CXC, CX_3C, and C) depending on the arrangement and number of the conserved N-terminal cysteine molecules. The CC chemokines (also called β chemokines) have the first 2 cysteine molecules adjacent, include regulated on activation normal T cells expressed and secreted (RANTES or CCL5), monocyte chemotactic protein-1 (MCP-1 or CCL2), macrophage inflammatory protein-1α (MIP-1α or CCL3) and MIP-1β (CCL4), and they preferentially attract monocytes and/or macrophages and eosinophils. The CXC chemokines (also called α chemokines) have the first 2 cysteine molecules separated by an amino acid, which includes interleukin (IL)-8 (or CXCL8), growth-related oncogene (GROα or CXCL1), and epithelial cell–derived neutrophil activating peptide (ENA-78 or CXCL5), and they preferentially attract neutrophils.5,6 Interferon-γ (IFNγ) has been demonstrated to modulate the release of chemokines in pneumococcal cell wall–stimulated mouse microglia differentially, supporting the view that activated microglia are a major intrinsic source of central nervous system (CNS) chemokine production and that IFNγ potentially regulates this microglial chemokine response pattern.7 Because chemokines are specific for particular cell types, the ratio and concentration of different chemokines in different body compartments determine the composition of the different cell types within the particular compartment. To understand the host response, IPD, more fully, it is important to gain insight into the production and regulation of chemokines at local sites of inflammation.

The HIV coreceptor CCR5 also acts as a receptor for CCL3, CCL4, and CCL5, and receptor binding and CCR5-dependent inhibition of HIV infection are induced by CCL8.8 CCR2b, the receptor for CCL2 (and CCL7, CCL8, and CCL13) is also a coreceptor for HIV and is expressed on peripheral blood lymphocytes and monocytes and/or macrophages.9 In HIV infection, CC chemokines induce an inflammatory response attracting noninfected target cells to the site of active viral replication and also induce intracellular signaling that enhances viral replication.10 The aims of this study were to investigate the chemokine response to IPD in children with pneumonia and meningitis and to examine the influence of HIV infection on the chemokine response, pneumococcal bacterial loads, and outcome.

METHODS

Study Participants

Study participants were recruited from Queen Elizabeth Central Hospital, Blantyre, southern Malawi, which serves a population of approximately 1 million. This is a government-funded teaching and referral hospital with 150 pediatric beds, although the number of pediatric inpatients is often in excess of 300. Children aged 2 months to 16 years identified as possibly having pneumonia (respiratory rate ≥50 breaths per minute for children <12 months

of age and ≥40 breaths per minute for children ≥12 months of age) or meningitis (stiff neck, bulging fontanelle, fever, and convulsions) were prospectively recruited into the study. Only children with confirmed disease were included in the analysis. The primary outcome measure was death or survival in the hospital.

Afebrile children without malaria parasitemia from the same villages as the patients were used as controls for plasma samples. Children admitted to the hospital for elective shunt surgery who had cerebrospinal fluid (CSF) microbiologic analysis before surgery and had no CSF pleocytosis were used as controls for CSF samples. It is unethical to perform a lumbar puncture on healthy children to obtain control CSF samples. We also included a control group of acutely ill children (HIV infected and HIV uninfected) without pneumococcal infection (and no other bacterial etiology detected), which allowed us to demonstrate that any differences in chemokine responses between HIV-infected and HIV-uninfected children were attributable to pneumococcal infection.

Ethical approval for this study was granted from the College of Medicine Research Committee (COMREC), Malawi, and the Liverpool School of Tropical Medicine Local Research Ethics Committee. Parents or guardians gave written informed consent for children to enter the study.

Definitions

- Confirmed pneumococcal pneumonia (n = 13): radiologic evidence of pneumonia (focal or lobar consolidation) plus 1 or more of the following: blood or lung culture aspirate positive for pneumococci and lung aspirate positive for pneumococcal polysaccharide antigen or pneumococcal DNA
- Confirmed pneumococcal meningitis (n = 82): abnormal CSF cell count >10 cells/μL plus 1 or more of the following: CSF culture positive for pneumococci, CSF Gram stain positive for pneumococci, CSF positive for pneumococcal polysaccharide antigen, and CSF positive for pneumococcal DNA
- Invasive pneumococcal disease (n = 95): *S pneumoniae* was identified (by culture, microscopy, antigen testing, or polymerase chain reaction [PCR]) from 1 or more of the following normally sterile body sites: blood, CSF, and lung aspirate
- Healthy controls (n = 10): healthy afebrile children from the same villages as the patients who had no malarial parasites on blood films
- CSF controls (n = 9): children admitted to the hospital for elective shunt surgery, with no CSF pleocytosis
- HIV-infected controls with no detectable bacterial infection (n = 22): HIV-infected children who presented with meningitis or pneumonia but were negative for any bacteria on culture, latex agglutination, and PCR (*S pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae*)
- HIV-uninfected controls with no detectable bacterial infection (n = 27): HIV-uninfected children who presented with meningitis or pneumonia but were negative for any bacteria on culture, latex agglutination, and PCR (*S pneumoniae*, *N meningitidis*, and *H influenzae*)
- Patients were categorized into “high responders” (CXCL8, CCL2, and CCL4 > median) and “low responders” (1 or more of the following: CXCL8, CCL2, and CCL4 ≤ median)
- Duration of symptoms before admission was classified as “long duration” (greater than median, 3 days) and “short duration” (≤3 days)

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Procedures

On admission, a complete history was taken, which included demographic details, previous medical history, vaccination status, and details of recent antibiotic use. Data were recorded on a specially designed proforma approved by both ethics committees. All children were fully examined and had their weight and height measured. All children had a blood sample taken for blood culture, a full blood count, malaria parasite analysis, blood glucose measurement, blood lactate measurement, HIV serology/PCR, pneumococcal PCR, and cytokine analysis. Children with suspected meningitis had a lumbar puncture performed, and CSF was taken for microscopy, culture, latex agglutination, pneumococcal PCR, and cytokine determination. Children with focal or lobar pneumonia had a lung aspirate performed in an area identified by a combination of chest radiographic findings and physical signs. The lung aspirate was sent for culture, microscopy, latex agglutination, and pneumococcal PCR.

Management Protocols

Children with meningitis were treated with 100 mg/kg of ceftriaxone administered intravenously once daily for 5 days. Thereafter, if a repeat lumbar puncture on day 2 did not show the continued presence of bacteria, they were recruited into a randomized controlled trial on day 6 to receive placebo or 100 mg/kg of ceftriaxone once daily for the next 5 days (10 days in total). Of all the children in this study who died, only 1 child was randomized into the trial. The rest were not randomized or died before randomization; therefore, the coexistence of a randomized controlled trial did not affect the main outcome parameter of this study. There were no children on antiretroviral therapy at the time of enrollment. HIV viral loads and CD4 cell counts were not routinely available.

The antibiotic regimen for pneumonia was intravenous benzyl penicillin (50,000 IU/kg every 6 hours) and gentamicin (6 mg/kg once daily) for at least 48 hours, with a switch to second-line therapy, intravenous cefuroxime (50 mg/kg every 8 hours) and gentamicin (6 mg/kg once daily), if no improvement was detected after 48 hours. On discharge, all children with pneumonia received oral amoxicillin to complete 10 days of treatment (125 mg every 8 hours for children <5 years of age and 250 mg every 8 hours for children >5 years of age).

Sample Processing

Whole blood collected in an ethylenediaminetetraacetic acid (EDTA) microtube was spun down at 3000 rpm for 10 minutes within 1 hour of collection, and the plasma was stored in aliquots for cytokine determination. Aliquots of whole blood (EDTA), CSF, and lung aspirate fluid were stored for quantitative real-time PCR of pneumococcal DNA, and whole blood (EDTA) and serum were stored for HIV analysis. All samples were stored at −80°C until analysis.

HIV Testing

HIV status was assessed in children ≥18 months of age using at least 2 of the following tests: Unigold (Trinity Biotech, Wicklow, Ireland), Serocard (Trinity Biotech), or Determine-HIV (Abbott Laboratories, Abbott Park, IL). In children less than 18 months of age and those with discordant antibody tests, HIV status was determined using the Amplicor HIV-1 DNA (Roche Diagnostics, Branchburg, NJ).

Development of Quantitative Real-Time Polymerase Chain Reaction for Pneumococcal DNA

Pneumococcal bacterial DNA was amplified and quantified using a real-time PCR assay as described previously.11 This assay selectively amplifies the pneumolysin (*ply*) gene, which encodes the hemolysin protein that is produced by all clinically relevant pneumococcal serotypes. In a typical real-time PCR assay, pneumococcal DNA from a patient sample was amplified using primers specific for the *ply* gene and a probe labeled with a reporter fluorescent dye (VIC, Applied Biosystems, Warrington, UK) and a quencher dye (TAMRA, Applied Biosystems, Warrington, UK). Accumulation of PCR products was measured directly by monitoring the increase in fluorescence of the reporter dye using the Applied Biosystems (Warrington, UK) 7700 real-time PCR instrument. Standards of known concentrations were amplified using the same real-time PCR assay to obtain cycle threshold (Ct) values at different concentrations. Ct values were then plotted against different serial dilutions of a pneumococcal control sample to construct a standard curve. The amount of bacterial DNA present in each sample was obtained by direct extrapolation of its Ct value to the amount of DNA (in copies/mL) as read from the concentration versus Ct standard curve.

Cytokine Determination

Multiple cytokine determination was performed in plasma and CSF samples using Luminex 100 technology (Luminex Corp., Austin, TX) in the Bioplex Protein Array System (Bio-Rad Laboratories, Hercules, CA). A 17-plex Bioplex Cytokine kit (Bio-Rad Laboratories) was used, which measures the following cytokines: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, CXCL8, IL-10, IL-12p70, IL-13, IL-17, granulocyte (G)-colony-stimulating factor, granulocyte macrophage (GM)-colony-stimulating factor, CCL2, CCL4, IFNγ, and tumor necrosis factor-α (TNFα). Each of the cytokines present in the reaction mixture was identified and quantified by the Bioplex Protein Array System based on bead color and intensity of the fluorescence signal produced. The concentration of each cytokine in the sample was automatically calculated from a standard curve derived from serial dilutions of the cytokine standard using BioPlex Manager software (Bio-Rad Laboratories). CCL5 was measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Quantikine; R&D Systems, Minneapolis, MN). A full set of chemokine data was available for 85 patients.

Statistical Analysis

The cytokine and bacterial DNA load data were not normally distributed; therefore, nonparametric statistical tests were used. Median levels of cytokine parameters were compared between 2 groups using the Mann-Whitney *U* test and between 3 groups using the Kruskal-Wallis test. Because the concentrations of pneumococcal DNA are exponential in nature, DNA loads were log-transformed (10-logarithm as the base), and associations between cytokines and log-transformed bacterial DNA loads were examined using the Spearman correlation coefficient. Paired data on CSF chemokines and cell counts were compared using the Wilcoxon signed rank test. Tests for association between binary variables were performed using the Fisher exact test. Multivariate logistic regression was used to explore risk factors for death. Variables were chosen based on biologic plausibility from the scientific literature, and those included in this stepwise analysis were plasma CCL2, CCL4, CCL5, and CXCL8; age; and duration of symptoms (each of which used on the original measurement scale) as well as HIV infection status, gender, previous antibiotic given or not, and meningitis or pneumonia (each a binary variable). The likelihood ratio was used to assess the significance of each variable. Reported *P* values are 2-tailed, and the 5% significance level was used to infer statistical significance. The Bonferroni correction was used (where applicable) to allow for the effects of multiple testing. The Statistical Package for Social Sciences (SPSS), version 13.0 (SPSS, Chicago, IL), was used for all analyses.

RESULTS

A total of 95 children with IPD were prospectively recruited, of whom 52 (54.7%) were male. There were 25 deaths (26.3%) before discharge, all of which occurred in the meningitis group (30.5%, n = 82) and none of which occurred in the pneumonia group (n = 13) ($P = 0.02$). There were 59 (62.1%) HIV-infected children, of whom 17 (28.8%) died, and 36 (37.9%) HIV-uninfected children, of whom 8 died (22.2%) ($P > 0.05$). The healthy controls were all HIV uninfected. In children with meningitis, 51 (62.2%) of 82 were HIV infected, and in children with pneumonia, 8 (61.5%) of 13 were HIV infected ($P > 0.05$).

CSF concentrations of CXCL8, CCL2, and CCL4 were all significantly higher than plasma concentrations, but CSF concentrations of CCL5 were significantly lower than those of plasma CCL5 ($P < 0.0005$). Plasma concentrations of CXCL8, CCL2, and CCL4 were significantly higher in patients than in healthy controls ($P = 0.001$, $P = 0.02$, and $P = 0.006$, respectively), but there was no significant difference in CCL5 concentrations. CSF concentrations of CXCL8, CCL2, and CCL4 were significantly higher in patients than in CSF controls ($P < 0.0005$, $P < 0.004$, and $P < 0.0005$, respectively); however, for CCL5, this difference was not significant. Plasma CXCL8, CCL2, and CCL4 were significantly higher in children with meningitis than in those with pneumonia ($P = 0.001$, $P < 0.0005$, and $P < 0.0005$, respectively), and CCL5 was lower, but this difference was not significant. Plasma concentrations of CXCL8 and CCL2 were statistically significantly higher in nonsurvivors than in survivors, but CCL5 was lower ($P = 0.02$, $P = 0.003$, and $P = 0.003$, respectively). CSF concentrations of CXCL8, CCL2, CCL4, and CCL5 were higher in nonsurvivors than in survivors, but this difference was only significant for CSF CCL5 ($P = 0.02$). Of the high responders, a higher proportion died (10 [35.7%] of 28) compared with low responders (9 [16.7%] of 54), but this difference was not significant.

Plasma and CSF concentrations of CXCL8, CCL2, CCL4, and CCL5S were higher in HIV-infected children versus HIV-uninfected children, but this was only significant for plasma CXCL8 ($P = 0.006$) and CCL2 ($P = 0.03$) and for CSF CXCL8 ($P = 0.006$) and CCL4 ($P = 0.01$) (Table 1). Median blood and CSF pneumococcal bacterial loads were higher in HIV-infected children compared with HIV-uninfected children: for blood, 2.07×10^3 DNA copies/mL versus 1.84×10^3 DNA copies/mL ($P > 0.05$), and for CSF, 1.05×50^8 DNA copies/mL versus 2.91×10^7 DNA copies/mL ($P = 0.04$). Median blood and CSF pneumococcal bacterial loads were also significantly higher in nonsurvivors compared with survivors: for blood, 6.32×10^3 DNA copies/mL versus 1.60×10^3 DNA copies/mL ($P = 0.02$), and for CSF, 1.47×70^8 DNA copies/mL versus 3.16×60^7 DNA copies/mL ($P = 0.03$). In HIV-infected children, plasma CXCL8 and CCL2 were significantly higher in nonsurvivors but plasma CCL5 was significantly lower, whereas in HIV-uninfected children, no significant differences were seen between survivors and nonsurvivors (Table 2).

Multivariate logistic regression analysis of the survival/nonsurvival outcome found evidence that male gender (odds ratio [OR] = 0.14, 95% confidence interval [CI]: 0.04 to 0.54) and lower plasma CCL2 levels (OR per unit reduction = 0.998, 95% CI: 0.996 to 0.999) are associated with an increased chance of survival. No evidence was found that any of the other variables considered (including HIV status and each of the cytokines) influenced the risk of survival after accounting for gender and CCL2 concentrations.

Because duration of symptoms may influence the chemokine profile and bacterial loads, we examined whether these parameters were influenced by duration of symptoms. There was no significant difference in duration of symptoms (as a continuous variable) between HIV-infected and HIV-uninfected children or between survivors and nonsurvivors. There were no significant differences in blood or CSF bacterial loads and chemokine concentrations

between those children with a long duration of symptoms compared with those with a short duration of symptoms. Similarly, there were no significant differences in blood or CSF bacterial loads and chemokine concentrations between children who had received antibiotics before admission and those who had not. A total of 35 (37%) of 95 children had antibiotics administered before admission, of whom 15 (43%) of 35 received parenteral antibiotics.

In HIV-infected children with IPD, median concentrations of all chemokines (except plasma CCL5, which was significantly decreased) were significantly increased compared with those in HIV-infected controls without detectable bacterial infection. Similarly, HIV-uninfected children with IPD had higher median chemokine concentrations (except plasma CCL5) than HIV-uninfected controls without detectable bacterial infection (see Table 1).

There were significant positive correlations between blood bacterial loads and plasma CXCL8, CCL2, and CCL4 and a significant negative correlation with CCL5 (Table 3). There were also significant correlations between CSF bacterial loads and CSF CXCL8, CCL2, CCL4, and CCL5 (see Table 3). In plasma, there was a weak negative correlation between CC chemokines and absolute blood lymphocyte count (CCL2: $r = -0.27$, $P = 0.05$; CCL4: $r = -0.36$, $P = 0.007$) but a stronger negative correlation between the CXC chemokine CXCL8 and absolute blood neutrophil count ($r = -0.71$, $P < 0.0005$).

In children with meningitis, we examined the correlation between CSF CXC chemokines and CSF neutrophils and CC chemokines and CSF lymphocytes, and there were no significant correlations. In view of the evidence, which suggests that IFNγ modulates the release of chemokines in pneumococcal cell wall–stimulated mouse microglia, we examined the correlation between CSF IFNγ and CSF cell counts. There was a weak negative correlation between CSF IFNγ and CSF neutrophil count ($r = -0.36$, $P = 0.006$) and CSF lymphocyte count ($r = -0.39$, $P = 0.003$). CSF neutrophil and lymphocyte counts were not significantly different between survivors and nonsurvivors, but in HIV-infected children, median CSF neutrophil and lymphocyte counts were lower than in HIV-uninfected children (524 vs. 792 cells/mm^3; $P = 0.06$ and 38 vs. 63 cells/mm^3; $P = 0.5$). Absolute blood neutrophil and lymphocyte counts were lower in HIV-infected children than in HIV-uninfected children ($P > 0.05$). Median CSF IFNγ concentrations were also significantly higher in HIV-infected children than in HIV-uninfected children (2048.37 vs. 1209.53 cells/mm^3; $P = 0.03$). Plasma and CSF IFNγ concentrations correlated strongly with plasma and CSF concentrations of CXCL8, CCL2, and CCL4 (see Table 3).

DISCUSSION

This prospective observational study examined the relation between chemokine responses in children presenting with pneumococcal meningitis and pneumonia and HIV status, pneumococcal bacterial loads, and outcome. HIV-infected children had higher chemokine concentrations and pneumococcal bacterial loads than HIV-uninfected children. The significant differences in plasma chemokines between survivors and nonsurvivors are largely seen in HIV-infected children. The number of HIV-uninfected children was small (n = 36), however, which may explain the failure to show a significant difference within this group. HIV-infected and HIV-uninfected children with IPD had higher median chemokine concentrations (except CCL5) than acutely ill HIV-infected and HIV-uninfected children with no detectable bacterial infection. It is well recognized that HIV-infected individuals have higher levels of chemokines; therefore, the inclusion of this control group should exclude the possibility that the higher levels seen in IPD may reflect little or no change from baseline.

Serum levels of CCL3 and CCL5, but not CCL4 have been shown to be increased in HIV-infected individuals compared with healthy controls,10 and plasma concentrations of CCL2, CCL3, and CCL5 were significantly higher in HIV-infected patients than in HIV-uninfected controls.12 One explanation for the finding of higher chemokines in HIV-infected children in our study might be that the concentrations detected are chronically, as opposed to acutely, elevated. This is an unlikely explanation, because HIV-infected children with pneumonia and meningitis in whom no bacterial infection was identified had significantly lower concentrations than those with confirmed IPD. Conversely, the higher number of chemokines in HIV-infected cases could reflect a florid host response secondary to the high pneumococcal bacterial loads, which would be supported by the strong correlations between pneumococcal bacterial loads and chemokine concentrations. High concentrations of CC and CXC chemokines in plasma and CSF coincident with lower CSF neutrophil and lymphocyte counts suggest a failure of leukocyte migration in response to cytokine stimulation, which might be attributable to impaired signaling mechanisms. HIV-1 Nef protein interferes with signaling pathways involved in T-cell activation and proliferation, resulting in an altered cellular response to antigen or cytokines,13 and HIV-1 inhibits GM-colony-stimulating factor activation of STAT5A, providing evidence of disruption of cellular signaling pathways after HIV-1 infection.14 Lipopolysaccharide and other microbial agents have been shown to cause a rapid and significant reduction of CCR2 (a receptor for CCL2) messenger RNA (mRNA) levels.15 Downregulation of CCR2 receptors in the presence of high cytokine concentrations might also explain the apparent failure of leukocyte migration.

The healthy controls included in this study were all HIV uninfected, and the HIV status of the CSF controls was unknown because these samples were taken anonymously from otherwise well children attending for elective shunt surgery. The control group of HIV-infected and HIV-uninfected children with no detectable bacterial infection is not ideal, because these children may have had acute viral infections or other bacteria that we were unable to detect. We did not have ethical approval to sample otherwise well HIV-infected children as an HIV-infected control group. Despite these limitations, our data confirm that the chemokine responses shown are associated with IPD, as opposed to a host response to HIV infection per se.

Multivariate regression analysis demonstrated that male gender and low plasma CCL2 concentrations are independently associated with survival. These findings are difficult to explain, but there is abundant evidence in the literature suggesting a key role for CCL2 in a variety of diseases characterized by monocyte and/or macrophage infiltration. Plasma CCL2 has been shown to correlate significantly with brain injury in HIV infection.16 Increased plasma levels of CCL2 may contribute to brain injury by influencing monocyte infiltration, which, in turn, may activate microglia and other macrophages, resulting in extensive injury to the brain and other tissues.16 Constitutive chemokine production may play a role in regulating inflammatory responses17 or in allowing immune surveillance to occur by increasing permeability of the blood-brain barrier.18 The mutant CCL2 allele (−2578G) has been linked to increased monocyte infiltration to tissues, increased serum CCL2 levels, and accelerated disease progression. It has been suggested that increased plasma CCL2 may confer partial protection from initial HIV infection but play a detrimental role after infection is established, contributing to accelerated disease progression and increased risk of HIV dementia.19 The risk associated with peripheral CCL2 levels may therefore depend on the degree of immunosuppression and other factors such as genotype, which were not examined in our regression model. The multivariate regression analysis allows evaluation of the significance of each variable after accounting for variation attributable to each of the others; therefore, colinearity of variables cannot be a reasonable explanation for this finding.

Neither duration of symptoms nor previous antibiotic administration seemed to have any effect on chemokine concentrations or pneumococcal bacterial loads. This can be explained by the fact that most children presented to the hospital late as well as by the fact that most of the prior antibiotic use was administered orally as opposed to parenterally.

We found substantially higher concentrations of the chemokines CXCL8, CCL2, and CCL4 in CSF than in blood, but the concentration of CCL5 was lower. This is in contrast to the findings of a study by Sprenger et al20 of 14 children with bacterial meningitis, where elevated chemokine levels were not found in the blood and CCL3 and CCL5 were not detected in the CSF. Our data are consistent with those of Spanaus et al,21 which showed significant elevations of chemokines in the CSF of patients with bacterial meningitis (10 with pneumococcal meningitis) compared with controls. Another consistent finding between this study and that of Spanaus et al21 is the lack of correlation between CSF mononuclear cells and CC chemokines and neutrophils and CXC chemokines. Our study reports a total of 82 children with confirmed pneumococcal meningitis, and therefore represents the largest clinical study to date examining the chemokine response in bacterial meningitis of a single etiology.

The CSF levels of CCL2 (but not of CCL3, CCL4, CXCL8, or CCL5) in HIV-infected patients with cytomegalovirus (CMV) encephalitis have been shown to be higher than those found in the CSF of HIV-infected patients with other neurologic diseases, suggesting that CCL2 production may underlie monocyte recruitment and tissue damage in CMV encephalitis.22 The higher CSF chemokine concentrations seen in HIV-infected children in this study might also represent a dysfunctional host response to pneumococcal infection of the CNS. The highly significant correlations between blood and CSF pneumococcal bacterial loads and blood and CSF chemokines, respectively, and higher CSF than plasma concentrations might argue for a compartmentalized response to invading pneumococci, but this study was not designed to investigate a causal relation.

The findings of a negative correlation between CSF IFNγ and CSF cell counts and a positive correlation between CSF IFNγ and CSF chemokines are interesting. The data from Hausler et al7 showed that IFNγ modulates cytokine and chemokine release from activated microglia in a complex but coordinated fashion. Pneumococcal cell wall components within the CSF (from pathogens crossing the blood-brain barrier, bacterial autolysis, and lysis after antibiotic treatment) are able to drive strong microglial activation, including synthesis of chemotactic factors.23 Hausler et al7 postulate that a specific feedback signal from invading leukocytes could affect microglial chemokine production, with changes in the proportions of chemokines altering the composition of cellular infiltrate, and that IFNγ could act as such a leukocyte-to-microglia signal. The negative correlation between IFNγ and CSF neutrophil and mononuclear cell counts would be consistent with a negative feedback mechanism; however, because IFNγ modulation of chemokine release is extremely complex, this could represent an incidental finding. The enhancement of CCL2 and CCL5 and suppression of CXCL1 and CXCL2 shown in Hausler et al's study7 would facilitate attraction of mononuclear cells and reduce chemotactic signals for neutrophils, which is what is seen in the course of bacterial meningitis. The exact role of IFNγ in regulating the chemokine response to pneumococcal infection still needs to defined.

Overall, survivors had lower chemokine concentrations than nonsurvivors, except for plasma CCL5, where higher concentrations were seen in survivors. When stratified by HIV status, the only statistically significant differences were seen in plasma CCL2, which was higher in nonsurvivors, and plasma CCL5, which was lower in nonsurvivors (correcting for multiple comparisons). These interesting findings are similar to those observed in children with meningococcal disease, where lower CCL5 concentrations were seen in children with

more severe disease and septic shock,24 and in a more recent study, where plasma concentrations of the chemokines CCL2, CCL3, and CXCL8 were significantly higher in fulminant meningococcal septicemia compared with mild systemic meningococcal disease but CCL5 was lower.25

In conclusion, this study has shown that in children with IPD, pneumococcal bacterial loads and chemokine concentrations are higher in HIV-infected children than in HIV-uninfected children. Chemokine concentrations are also generally higher in HIV-infected and HIV-uninfected children with IPD compared with acutely ill controls with no detectable bacterial infection. Chemokine concentrations correlate with pneumococcal bacterial loads and outcome. The findings in HIV-infected children suggest an impaired cellular response to chemoattractant cytokines.

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TABLE 1

Median Plasma Chemokine Concentrations in HIV-Infected and HIV-Uninfected Children With IPD and No Bacterial Infection Detectable

	HIV Infected (n = 81) Median			HIV Uninfected (n = 73) Median			
Parameter	**IPD (n = 59)**	**NBI (n = 22)**	***P***	**IPD (n = 36)**	**NBI (n = 27)**	**Controls (n = 10)**	***P***
Plasma CXCL8 (pg/mL)	88.58	23.79	0.003 *	29.37	10.42	2.23	0.001 *†
							0.006 *‡
							0.006 *§
Plasma CCL2 (pg/mL)	89.62	21.95	0.001 *	53.23	20.48	1.23	0.006 *†
							NS ‡
							0.03 §
Plasma CCL4 (pg/mL)	335.40	76.67	<0.0005 *	184.19	50.29	76.37	<0.0005 *†
							<0.0005 *
							NS §
Plasma CCL5 (pg/mL)	25394.90	46671.25	0.045	14543.10	26814.70	41250.05	NS †
							NS ‡
							NS §
CSF CXCL8 (pg/mL)	97481.22	2133.16	0.004 *	32293.62	127.13	65.32//	<0.0005 *†
							<0.0005 *‡
							0.006 *§
CSF CCL2 (pg/mL)	55746.78	404.15	0.025	39623.11	170.86	2750.98//	0.002 *†
							0.001 *‡
							NS §
CSF CCL4 (pg/mL)	26527.44	191.29	0.005 *	8356.49	2.51	31.78//	<0.0005 *†
							0.001 *‡
							0.01 §
CSF CCL5 (pg/mL)	116.90	ND	0.041	38.45	ND	46.00//	NS †
							NS ‡

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	HIV Infected (n = 81) Median			HIV Uninfected (n = 73) Median			
Parameter	**IPD (n = 59)**	**NBI (n = 22)**	***P***	**IPD (n = 36)**	**NBI (n = 27)**	**Controls (n = 10)**	***P***
							NS[§]

Values are median concentrations and IQR.

IQR indicates interquartile range; NBI, no detectable bacterial infection; ND, not detected; NS, not significant.

[*] Statistically significant result using Bonferroni correction of multiple testing.

[†] Kruskal-Wallis statistic for comparison between IPD, NBI, and controls.

[‡] Mann-Whitney *U* statistic for comparison between IPD and NBI.

[§] Mann-Whitney *U* statistic for comparison between HIV-infected and HIV-uninfected children with IPD.

[//] CSF controls (anonymous [see text]; therefore, HIV status unknown).

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TABLE 2

Comparison of Median Plasma and CSF Chemokine Concentrations in Survivors and Nonsurvivors by HIV Infection Status

	Parameters	Survivors (n = 70) Median (IQR)	Nonsurvivors (n = 25) Median (IQR)	*P*
HIV infected (n = 59)	Number	42	17	
	Plasma CXCL8 (pg/mL)	49.04 (22.60–246.31)	159.28 (53.77–1506.73)	0.03
	Plasma CCL2 (pg/mL)	43.90 (25.01–276.51)	536.75 (53.25–1495.05)	0.002 *
	Plasma CCL4 (pg/mL)	248.57 (103.55–708.52)	626.01 (265.01–1026.31)	NS
	Plasma CCL5 (pg/mL)	38885.40 (20,690.53–79,817.93)	11790.00 (6609.53–22,541.85)	<0.0005 *
	CSF CXCL8 (pg/mL)	92745.58 (26,687.67–133,438.10)	105125.90 (50,059.85–127,617.80)	NS
	CSF CCL2 (pg/mL)	44374.22 (7296.23–102,695.78)	145664.05 (45,726.42–216,549.83)	NS
	CSF CCL4 (pg/mL)	24658.24 (8378.61–47,334.08)	33549.73 (12,002.73–50,717.90)	NS
	CSF CCL5 (pg/mL)	159.20 (44.35–437.55)	211.50 (111.00–861.30)	NS
HIV uninfected (n = 36)	Number	28	8	
	Plasma CXCL8 (pg/mL)	29.34 (16.84–62.82)	52.03 (14.72–212.87)	NS
	Plasma CCL2 (pg/mL)	48.59 (15.69–109.25)	80.95 (19.08–252.44)	NS
	Plasma CCL4 (pg/mL)	171.46 (105.81–343.18)	270.63 (184.19–311.46)	NS
	Plasma CCL5 (pg/mL)	14543.10 (8333.93–46,072.90)	24918.85 (6261.26–57,404.50)	NS
	CSF CXCL8 (pg/mL)	35317.24 (17,926.18–89,573.15)	12765.11 (3583.31–54,666.39)	NS
	CSF CCL2 (pg/mL)	39623.11 (1652.77–65,630.45)	24853.41 (183.15–65,754.35)	NS
	CSF CCL4 (pg/mL)	10025.39 (5539.22–31,908.47)	4910.69 (1096.53–9437.13)	NS
	CSF CCL5 (pg/mL)	58.90 (29.70–177.80)	211.50 (111.00–861.30)	NS

Values are median concentrations and IQR.

IQR indicates interquartile range; NS, not significant.

* Statistically significant result using Bonferroni correction of multiple testing.

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TABLE 3

Spearman Correlations Between Plasma and CSF Chemokines and Blood and CSF Pneumococcal Bacterial Loads and Blood and CSF IFNγ Concentrations, Respectively

	Correlation Between Blood Bacterial Load and Plasma Chemokines	*P*	Correlation Between Plasma IFNγ and Plasma Chemokines	*P*
Plasma CXCL8 (pg/mL)	0.42	<0.0005 *	0.61	<0.0005 *
Plasma CCL2 (pg/mL)	0.47	<0.0005 *	0.65	<0.0005 *
Plasma CCL4 (pg/mL)	0.55	<0.0005 *	0.48	<0.0005 *
Plasma CCL5 (pg/mL)	−0.33	0.003 *	0.01	NS
	Correlation Between CSF Bacterial Load and CSF Chemokines		**Correlation Between CSF IFNγ and CSF Chemokines**	
CSF CXCL8 (pg/mL)	0.50	0.003 *	0.44	<0.0005 *
CSF CCL2 (pg/mL)	0.61	0.001 *	0.38	0.01
CSF CCL4 (pg/mL)	0.54	0.001 *	0.48	<0.0005 *
CSF CCL5 (pg/mL)	0.43	0.03	0.32	NS

Spearman correlation coefficient is used.

NS indicates not significant.

* Statistically significant result using Bonferroni correction of multiple testing.