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MAJOR ARTICLE

Human Immune Response Varies by the Degree of Relative Cryptococcal Antigen Shedding

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Background. Cerebrospinal fluid (CSF) cryptococcal glucuronoxylomannan antigen (CrAg) titers generally correlate with quantitative fungal culture burden; however, correlation is not precise. Some patients have higher CrAg titers with lower fungal burdens and vice versa. We hypothesized that the relative discordancy between CrAg titer and quantitative culture burden reflects the relative degree of CrAg shedding by *Cryptococcus neoformans* and is associated with human immune responses.

Methods. One hundred ninety human immunodeficiency virus-infected individuals with cryptococcal meningitis were enrolled in Uganda and South Africa. We compared initial CSF CrAg titers relative to their CSF quantitative cultures to determine low (n = 58), intermediate (n = 68), or high (n = 64) CrAg shedders. We compared cytokines measured by Luminex multiplex assay on cryopreserved CSF and 10-week mortality across shedding groups using linear and logistic regression and distribution of genotypes by multilocus sequence typing.

Results. The relative degree of CrAg shedding was positively associated with increasing CSF levels of the following: interleukin (IL)-6, IL-7, IL-8, and tumor necrosis factor-α (each $P < 0.01$), which are all secreted by antigen-presenting cells and negatively associated with vascular endothelial growth factor ($P = .01$). In addition, IL-5, IL-13, granulocyte colony-stimulating factor, and macrophage chemotactic protein were decreased in low-CrAg shedders compared with intermediate shedders (each $P \leq .01$). Type 1 T-helper cells (Th1) cytokine responses and 10-week mortality did not differ between the shedding groups. Cryptococcal genotypes were equally distributed across shedding groups.

Conclusions. Discordancy between CrAg shedding and expected shedding based on quantitative fungal burden is associated with detectable immunologic differences in CSF, primarily among secreted cytokines and chemokines produced by antigen-presenting cells and Th2.

Keywords. cerebrospinal fluid; cryptococcal meningitis; *Cryptococcus*; HIV/AIDS; immune response.

In Africa, cryptococcal meningitis is the most common cause of adult meningitis and is responsible for 15%–20% of acquired immune deficiency syndrome (AIDS)-related mortality [1–3]. Survival after cryptococcosis in sub-Saharan Africa is often ≤40% [3, 4]. Quantification of the burden of cryptococcal infection can be assessed by quantitative cerebrospinal fluid (CSF) culture or semiquantitative cryptococcal antigen (CrAg) titer. Higher burdens of infection are associated with higher mortality [5, 6]. These quantitative methodologies correlate generally but imperfectly. Some consider such discordancy as random measurement error. However, we hypothesized that different degrees of cryptococcal glucuronoxylomannan (GXM) polysaccharide shedding in the CSF may modulate the human immune responses to *Cryptococcus neoformans* to impact mortality.

As evidenced by the high rate of cryptococcal meningitis in persons living with AIDS, $CD4^+$ T helper cells (Th) play a critical role in combating *Cryptococcus* infections. Developing a robust Th1 cell-mediated immune response against *Cryptococcus* is protective [7–9], but murine model evidence suggests that *Cryptococcus* can subvert the host immune response through its GXM polysaccharide capsule [10–12]. In contrast, a Th2-mediated response promotes increased disease pathology and promotes mortality in murine models [12, 13]. Human data are more limited. In vivo, there can be substantial interperson and intraperson variation in capsule morphology and diameter of *Cryptococcus* strains [14]. In addition, the human immune response and survival can be influenced by *Cryptococcus* strain variation [15]. Jarvis et al [9] found that higher CSF levels of interferon-γ, interleukin (IL)-6, IL-4, IL-10, and IL-17 correlated with more rapid clearance of *Cryptococcus* from the CSF and improved 2-week survival.

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Due to the cryptococcal capsule's integral role in pathogenicity and evasion, we hypothesized that the relative degree of capsule GXM shedding (ie, CrAg titer) for any given quantitative CSF culture burden is associated with the human immune response and impacts clinical outcome.

METHODS

Study Population

The study population consisted of 190 human immunodeficiency virus (HIV)-infected, antiretroviral therapy (ART)-naive individuals with a first episode of cryptococcal meningitis screened for the Cryptococcal Optimal ART Timing (COAT) trial (clinicaltrials.gov: NCT01075152) [16]. Participants were enrolled from Mulago Hospital in Kampala, Uganda, Mbarara Hospital in Mbarara, Uganda and GF Jooste Hospital in Cape Town, South Africa between November 2010 and April 2012. Each participant provided written informed consent upon screening for the trial, and institutional review board approvals were obtained.

Diagnostic Testing

Cerebrospinal fluid quantitative fungal burden was assessed by plating 100 μL of CSF in four 1:10 serial dilutions on Sabouraud dextrose agar [17]. Cerebrospinal fluid cultures were incubated for up to 14 days; the highest dilution plates with discrete colonies were used to calculate colony-forming units (CFU) per milliliter of CSF. The CrAg lateral flow assay (CrAg LFA; Immy, Inc., Norman, OK) was used to determine CrAg titer. The CrAg LFA uses gold nanoparticle-conjugated monoclonal antibodies specific for GXM, the primary capsule polysaccharide, of all 4 serotypes of Cryptococcus (A–D) [6]. Qualitative measurements were done onsite according to manufacturer's instructions. Semiquantitative CrAg titers were assessed on cryopreserved (−80°C) CSF by initial dilution of 1:25 followed by 1:2 serial dilutions in a 96-well plate and titer determined as the last visually positive strip [18]. If a sample was negative at 1:25, serial dilutions were run on an initial dilution of 1:2. Samples were assayed at 25°C using manufacturer provided titration diluent and read after 10 minutes.

Cytokine Analysis

Cytokine analysis was conducted on cryopreserved CSF. Nineteen cytokines were assayed in duplicate using the Bio-Rad Bio-Plex Human Cytokine 17-Plex Panel (Bio-Rad, Austin, TX). Vascular endothelial growth factor (VEGF) and macrophage inflammatory protein-1α (MIP-1α [CCL3]) were introduced later in the analysis with only 126 and 85 patients, respectively. Cerebrospinal fluid was snap thawed in a 25°C water bath to preserve protein integrity and diluted 1:4 in a separate 96-well plate according to manufacturer's instructions and assayed via the Bio-Rad Luminex 100 system.

Cytokine results were determined using the Bio-Plex Manager software. "Out of range" measurements were set to the lowest detectable standard divided by 2. Values above the highest standard were set to 10% above the highest standard value. All cytokine values were $\log_2$ transformed for analysis and then back-transformed to present the geometric mean [19].

Multilocus Sequence Typing

Eight gene loci were amplified, sequenced, and analyzed as previously described [15]. Genomic DNA was extracted [20], after which 8 loci were amplified and sequenced, including 7 International Society for Human and Animal Mycology consensus loci (CAP59, GPD1, IGS1, LAC1, PLB1, SOD1, and URA5) and the optional TEF1 locus [21, 22]. Locus alleles and subsequent sequence types were numbered based on the Fungal MLST Database for *C neoformans* (mlst.mycologylab.org), and novel alleles and sequence types were deposited into the database.

Statistical Analysis

To differentiate between high CrAg titer due to high fungal burden and strains with high capsule shedding, we examined the following: (1) the relative shed capsule normalized to initial quantitative culture, (2) the absolute CSF CrAg titer, and (3) the absolute quantitative CSF culture. Cryptococcal antigen shedding categories were defined by approximate tertiles of the observed-to-predicted CrAg titer difference based on a linear relationship of $\log_2$ CSF quantitative culture and $\log_2$ CrAg titers. Participants greater or less than 15% from the predicted CrAg values were classified as high and low shedders, respectively. Baseline characteristics were compared with χ^2 tests and Kruskal-Wallis tests, as appropriate. Multilocus sequence typing (MLST) genotypes were compared with χ^2 tests. Cytokine levels in each category were compared using linear regression models, with intermediate shedders as reference for pairwise comparisons. Cytokine models were repeated with adjustment for CSF leukocyte count, because CSF leukocyte count was associated with shedding group. Associations with mortality and CrAg shedding categories were determined with logistic regression models.

RESULTS

Among 237 patients with cryptococcal meningitis screened for the COAT trial, 190 had CSF stored and matched *C neoformans* isolates available for analysis in this prospective substudy. To compare capsule shedding to clinical factors, the amount of capsule shedding was categorized as low (n = 58), intermediate (n = 68), or high (n = 64) using CSF CrAg titer relative to the quantitative CSF culture (Figure 1). No association was found between the relative CrAg shedding groupings and age, sex, country of origin, Glasgow Coma Score, CD4 count, HIV viral load, CSF opening pressure, or CSF quantitative culture (Table 1).

Relative CrAg shedding was associated with the CSF immune response. Persons with lowest tertile of shedding had the highest median CSF white blood cell count although generally lower soluble cytokine responses. In comparing CSF immune response with CrAg shedding, 16 cytokines/chemokines had

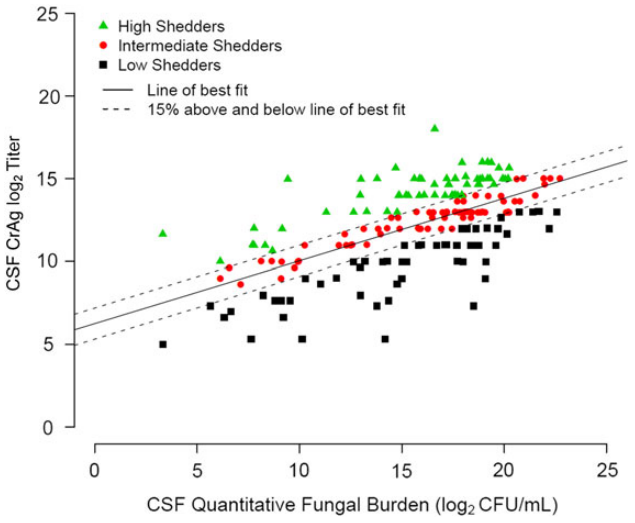

Figure 1. Cerebrospinal fluid (CSF) cryptococcal antigen (CrAg) shedding in comparison with CSF quantitative culture. The graphic displays CSF CrAg titer vs CSF quantitative fungal culture with $\log_2$ transformation. Cryptococcal Ag-shedding categories are delineated 15% above and below the average best-fit line (Pearson correlation coefficient of 0.64), forming 3 categories of low, intermediate, and high relative CrAg shedding. Cytokine analysis and clinical correlates were determined using these 3 relative shedding groups.

routinely measureable CSF levels. A positive association with CrAg shedding existed for IL-6, IL-7, IL-8, tumor necrosis factor-α (TNF-α), granulocyte-colony stimulating factor (G-CSF), IL-13, and monocyte chemoattractant protein-1 (CCL2) (each $P \leq .01$) whereby persons with higher relative CrAg shedding had higher CSF cytokine/chemokine levels (Table 2). There was a negative association with CrAg shedding status and VEGF ($P = .01$). More intricate trends were revealed when we used linear regression pairwise comparisons with the intermediate CrAg shedders as the reference group (Table 3). The statistical differences in IL-7, IL-13, G-CSF, TNF-α, and CCL2 were attributed to low relative CrAg shedders having lower levels than intermediate CrAg shedders. There were few differences between intermediate and high relative CrAg shedders; the only significant difference was IL-17 (geometric mean: 10 pg/mL vs 18 pg/mL, respectively; $P = .01$). Three cytokines had high proportions of undetectable measurements (IL-1β, IL-2, and IL-5) and were considered as detectable versus undetectable responses. Interleukin-5, a Th2 cytokine, was detectable in 40% of low shedders, 63% of intermediate shedders, and 66% of high relative CrAg shedders ($P < .01$).

The association between mortality and degree of CrAg shedding was assessed in 173 participants with known outcome. Mortality within 10 weeks of cryptococcal meningitis diagnosis was 34% (17 of 50) for the low CrAg relative shedding group, 46% (31 of 67) for the intermediate shedding group, and 45% (25 of 56) for high shedding group. The odds of death was nonstatistically higher among the intermediate and high CrAg relative shedding groups compared with the low shedders (odds ratio = 1.6; 95% confidence interval, .8–3.2; $P = .17$). Thus, whereas the degree of relative CrAg shedding was associated with the initial host immune response, the degree of relative CrAg shedding was not associated with survival. In contrast, the increasing absolute CSF fungal burden was associated with increasing mortality with low tertile of CSF culture having a 10-week mortality of 31% (17 of 55), intermediate tertile 40% (23 of 57), and high terile 54% (33 of 61) ($P = .04$).

When comparing CSF immune responses to either the absolute fungal burden by culture or absolute CrAg titer, there were fewer apparent differences than when considering the relative CrAg shedding. There was minimal correlation between the CSF immune response and the absolute quantitative CSF fungal culture burden at diagnosis (Supplementary Table 1). The absolute CrAg titer, when grouped by tertiles, had positive

Table 1. Baseline Characteristics and Clinical Outcomes by Degree of Cryptococcal Antigen Relative Shedding[a]

Baseline Characteristic	N With Data	CrAg Relative Shedding Status			*P* Value
		Low N = 58	Intermediate N = 68	High N = 64	
Age, years	190	33 (27, 40)	37 (30, 42)	37 (29, 40)	.11
Male sex, N (%)	190	28 (48%)	36 (53%)	36 (56%)	.68
Glasgow Coma Score <15, N (%)	190	18 (31%)	26 (38%)	17 (27%)	.35
CD4 T cell count/μL	151	17 (9, 79)	36 (10, 76)	30 (13, 72)	.82
HIV RNA, $\log_{10}$ copies/mL	151	5.4 (5.1, 5.7)	5.5 (5.1, 5.9)	5.6 (5.4, 5.8)	.08
CSF white cell count/μL	145	50 (<5, 155)	30 (<5, 125)	10 (<5, 45)	.05
CSF white cell <5 cells/μL, N (%)	145	17 (40.5%)	16 (28%)	18 (40%)	.30
CSF opening pressure, mmH_2O	164	260 (200, 380)	310 (190, 440)	250 (175, 350)	.36
CSF quantitative culture, $\log_{10}$ CFU/mL	190	4.6 (3.8, 5.6)	5.2 (4.0, 5.7)	5.3 (4.5, 5.6)	.34
Clinical Outcomes					
CSF yeast clearance rate, $\log_{10}$ CFU/mL per day, mean (95% CI)	154	0.34 (0.29–0.40)	0.33 (0.29–0.36)	0.28 (0.24–0.32)	.39
10-week mortality, N (%)	173	17 (34%)	31 (46%)	25 (45%)	.37

Abbreviations: CFU, colony-forming units; CI, confidence interval; CrAg, cryptococcal antigen; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; IQR, interquartile range.

[a] Data are median (IQR) or N (%). Cerebrospinal fluid clearance is mean (95% CI) as calculated by mixed-effects model.

Table 2. Cerebrospinal Fluid Cytokine Profiles by Degree of Cryptococcal Antigen Relative Shedding[a]

CSF Biomarker, pg/mL	N With Data	CrAg Relative Shedding Status			*P* Value
		Low N = 58	Intermediate N = 68	High N = 64	
IL-4	190	0.80 (0.62, 1.03)	0.80 (0.63, 1.01)	0.95 (0.74, 1.21)	.53
IL-6	190	87.3 (50.2, 152)	193 (116, 322)	295 (174, 499)	<.01
IL-7	190	2.8 (2.0, 3.7)	5.5 (4.1, 7.3)	6.6 (4.9, 8.8)	<.001
IL-8	190	476 (353, 642)	640 (485, 844)	911 (685, 1212)	<.01
IL-10	190	6.9 (5.4, 8.7)	7.7 (6.2, 9.6)	9.6 (7.6, 12.0)	.13
IL-12	190	6.4 (4.9, 8.5)	10.1 (7.8, 13.0)	9.0 (6.9, 11.7)	.06
IL-13	190	10.4 (6.2, 17.2)	26.6 (16.6, 42.7)	27.4 (16.8, 44.4)	<.01
IL-17	190	10.5 (7.2, 15.3)	9.8 (6.9, 13.9)	18.3 (12.8, 26.2)	.03
G-CSF	190	49.3 (36.9, 65.8)	86.1 (65.9, 112)	91.1 (69.2, 120)	<.01
GM-CSF	190	358 (293, 436)	269 (224, 323)	300 (249, 363)	.12
Interferon-γ	190	28.3 (19.3, 41.6)	43.0 (30.2, 61.4)	43.0 (29.8, 62.0)	.20
TNF-α	190	8.3 (5.8, 11.8)	17.1 (12.3, 23.6)	27.5 (19.7, 38.4)	<.001
CCL2 (MCP-1)	190	331 (238, 460)	642 (474, 871)	724 (529, 991)	<.01
CCL3 (MIP1α)	85	10.8 (4.7, 24.9)	16.7 (9.3, 29.9)	30.0 (17.1, 52.4)	.11
CCL4 (MIP1β)	190	60.0 (43.1, 83.6)	69.8 (51.4, 94.8)	54.7 (39.9, 74.9)	.54
VEGF	126	49.1 (26.2, 92.1)	24.0 (14.9, 38.8)	14.7 (9.0, 24.1)	.01

Abbreviations: CCL2 (MCP-1), monocyte chemoattractant protein-1; CCL3 (MIP1-α), macrophage inflammatory protein-1α; CCL4 (MIP-1β), macrophage inflammatory protein-1β; CrAg, cryptococcal antigen; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-monocyte CSF; IL, interleukin; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

[a] Values are geometric mean (with 95% confidence interval) in pg/mL by group. *P* values are from general linear models.

associations with IL-7, IL-17, TNF-α, CCL2, and MIP1α (Supplementary Table 2). CD4 T-cell count in blood was inversely associated with both absolute CSF quantitative culture burden (*P* < .001). Those with the lowest fungal burden (CSF culture <26 000 CFU/mL) had the highest median CD4 count of 62 cells/μL (interquartile range [IQR], 15–103) compared with those with intermediate fungal burden, whose CD4 count was 32 (IQR, 11–72) cells/μL, and those with the highest fungal burden (CSF culture >262 000 CFU/mL) had a CD4 count of 14 (6–37) cells/μL. Overall, the relative capsule shedding had more associations with CSF immune response than the absolute CrAg titer and more extreme statistical differences.

Table 3. Relative Difference in Cerebrospinal Fluid Cytokine Response by Degree of Cryptococcal Antigen Relative Shedding[a]

CSF Biomarker	Intermediate vs Low CrAg Shedders		High vs Intermediate CrAg Shedders	
	% Geometric Difference	*P* Value	% Geometric Difference	*P* Value
IL-4	0.3%	.99	18%	.33
IL-6	121%	**.04**	53%	.26
IL-7	99%	**<.01**	20%	.37
IL-8	34%	.15	42%	.08
IL-10	12%	.50	24%	.18
IL-12	57%	**.02**	−11%	.53
IL-13	157%	**<.01**	3%	.94
IL-17	−6.6%	.79	86%	**.01**
G-CSF	74%	**<.01**	6%	.77
GM-CSF	−25%	**.04**	12%	.41
Interferon-γ	52%	.12	0%	.99
TNF-α	106%	**<.01**	61%	.05
CCL2 (MCP-1)	94%	**<.01**	13%	.59
CCL3 (MIP1α)	55%	.40	80%	.15
CCL4 (MIP1β)	16%	.51	−22%	.27
VEGF	−51%	.08	−39%	.16

Abbreviations: CCL2 (MCP-1), monocyte chemoattractant protein-1; CCL3 (MIP1-α), macrophage inflammatory protein-1α; CCL4 (MIP-1β), macrophage inflammatory protein-1β; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-monocyte CSF; IL, interleukin; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor. Bolded text indicates significance at the 0.05 level.

[a] Relative difference in $\log_2$ geometric mean values, where 0% difference is equal value and 100% is a 2-fold increase. Analysis is by pairwise comparison.

We also assessed whether strain genotype was associated with the relative degree of CrAg shedding. No relative shedding group was overrepresented in any of the burst groups (Figure 2). The most common sequence type observed in the cohort (ST93, n = 61) compared with all other sequence types also revealed no significant difference in the distribution between shedding group among those with ST93 genotype or without (χ^2 test, *P* = .23).

DISCUSSION

In this study comparing localized CSF immune responses with the degree of *Cryptococcus* capsular GXM polysaccharide shed, we show that higher relative capsule shedding was associated with a proinflammatory response as well as a greater Th2-mediated immune response. Multiple proinflammatory cytokines and chemokines (eg, IL-6, IL-8, TNF-α, CCL2) produced by antigen-presenting cells were increased among persons that had higher degrees of relative capsule shedding, as were Th2 cytokines (eg, IL-5, IL-13) produced by Th2 cells. There was no difference in interferon-γ levels (Th1 cytokine) based on

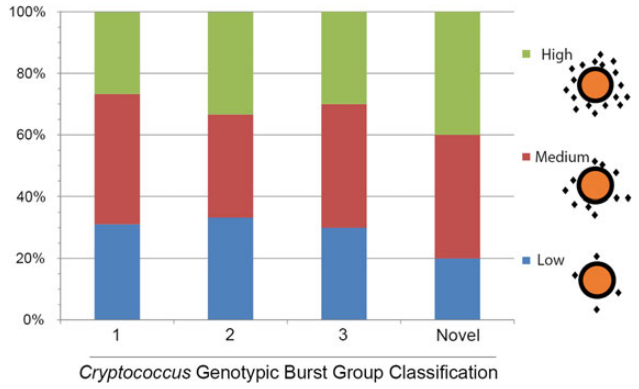

Figure 2. Proportion of relative cryptococcal antigen (CrAg) shedding by *Cryptococcus* genotype multilocus sequence typing grouping. The graphic displays the proportion of high, medium, low relative CrAg shedders categorized into genotype burst group of Ugandan isolates, as defined in Wiesner et al [15]. Novel isolates that did not fall into the prior burst group 1, 2, or 3 groupings are categorized as "novel." Genotype data included 146 isolates for group 1 (n = 78), group 2 (n = 15), group 3 (n = 15), and novel (n = 78). In the prior genotype analysis, burst group 1 had increased lethality among humans infected with this *Cryptococcus neoformans* var. *grubii* genotype in Uganda. Herein, there was no association between the degree of relative CrAg shedding and genotype.

relative capsule shedding, absolute CrAg titer, or culture burden. The low relative capsule shedding group's immune response was different than the other 2 groups and drove most of the statistical findings in this study. The high capsule shedding group was quite similar to the intermediate shedding group, except for approximately 2-fold higher IL-17 CSF levels. This positive association with the degree of relative capsule shedding is in marked contrast with the negative correlation in the immune response observed with increasing capsule thickness observed in the same cohort [14]. Increased capsule thickness was associated with decreased interferon-γ, IL-4, IL-6, IL-7, IL-8, and overall CSF white cell counts among participants in Kampala, Uganda [14]. Taken together, these data support the hypothesis that increased capsule shedding results in increased inflammation and a more pronounced Th2-mediated immune response.

An alternative explanation is that an immune response skewed toward a nonprotective Th2 response allows for uncontrolled infection and results in greater absolute amounts of capsule production [13]. Analysis of high/medium/low absolute quantitative culture alone showed no significant associations with immune response, indicating that the key variable was the amount of shed capsule generated per CFU. Absolute shed capsule, ie, CrAg titer, is reflective of burden of disease and is associated with survival [6]. In this prospective substudy, we cannot control for time to patient presentation or delay in accessing healthcare. Thus, we are unable to determine the dynamics of the relationship between quantitative culture and CrAg titer and can envision at least 2 infection scenarios. First, slow growing strains could generate higher amounts of shed capsule due to a longer time to clinical presentation. Alternatively, some strains may shed more capsule that other strains. Previous studies have shown an association between amount of in vitro capsule shedding and clinical parameters of disease [14], suggesting the latter scenario can occur.

Statistically significant differences were noted in CCL2, G-CSF, and IL-13, and positive correlations with shedding among IL-6, IL-7, IL-8, and TNF-α (secreted by antigen-presenting cells) levels in low shedders versus intermediate shedders. It is interesting to note that Jarvis et al [9] found higher levels of IL-6, IL-4, IL-10, IL-17, and interferon-γ correlated with increased macrophage activation, more rapid clearance of *Cryptococcus* from the CSF, and improved 2-week survival. The majority of these cytokines (eg, IL-4, IL-10, IL-17, and interferon-γ) are produced by T cells, indicating that a better T-cell immune response was protective overall. Jarvis et al [9] also found that increased CCL2 and MIP1α correlated with increased risk of immune reconstitution inflammatory syndrome (IRIS). With this in mind, the immune response associated with relative capsule shedding tertiles observed in our study did not clearly follow the categories put forth by Jarvis et al [9]. Instead, our signature seems to be a combination of that observed by Jarvis et al [9] and recent studies examining the role of cell wall chitin in generation of Th2-mediated immune responses [12]. Strains with high capsule shedding have reduced capsule size [14], and these strains may have increased exposure of cell wall structures such as chitin that can stimulate additional immune responses [12]. Our data suggest subversion of the immune response may reflect on the relative degree of capsule shedding.

A fundamental unanswerable question within this cohort study is whether the human immune response provokes an alteration in cryptococcal gene expression, or if the organism manipulates the human immune response. In the setting of advanced AIDS, the immunocompromised human immune response is dysfunctional and perhaps easily subverted by an opportunistic pathogen such as *Cryptococcus*. Higher rates of capsule shedding could provoke a pro-inflammatory response from the innate immune system's antigen-presenting cells, which are still functional despite HIV/AIDS. Furthermore, strain-specific capsule differences can cause a skewed, nonproductive Th2 immune response [15].

It is interesting to note that cryptococcal genotype assessed by MLST was not associated with variations in the capsule shedding phenotype. A number of possibilities could explain this result. The sample size may have been underpowered to identify differences due to a large proportion of the population containing the ST93 or closely related genotypes. It is also possible that genetic loci impacting capsule shedding are independent of the population structure depicted by MLST genotyping. Alternatively, the capsule shedding phenotype may be dependent upon the host environment instead of a distinct genetic DNA signature. Whole genome sequencing and single nucleotide

polymorphism analysis would be needed to determine whether this phenotype is genetically linked or whether the in vivo capsule shedding is a response to the host environment. Further explanation of the cause-effect directionality between genotype, immune response, and capsule shedding needs to be explored via murine experimental models combined with whole genome sequencing and gene expression studies.

Many studies have linked paucity of inflammatory responses to poor clinical outcome and increased mortality [9, 23, 24]. In this study, the low capsule shedders had less inflammation, and there was a trend towards increased mortality among the intermediate and high shedders compared with the low capsule shedders. Because there are other AIDS-related and nosocomial causes of mortality beyond cryptococcosis [16], this comparison is likely biased to the null, and a larger sample size may have revealed a statistical difference. This is an important question because the ability to shed CrAg may influence clinical status. The inability to clear organisms or high-level antigenemia is a risk factor for paradoxical IRIS and is a marker of a dysfunctional immune system [19, 25]. Furthermore, given that immunologic differences are clearly present, lower shed antigen levels may lead to restoration of immune homeostasis and decreased harmful Th2 responses, providing a better environment for responding to further opportunistic infections to which persons with advanced HIV and cryptococcal meningitis are extremely susceptible.

CONCLUSIONS

This study highlights a very important aspect of cryptococcal infection: the complex interactions between the human host, the infecting strain, and clinical disease progression that ultimately culminate in patient outcome. Our results, demonstrating that high amounts of capsule shedding are associated with inflammation and Th2-mediated responses, provide additional evidence for the interplay between pathogen and host and highlight the need for treatment strategies aimed at both killing the pathogen and monitoring and modulating the host immune response.

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

Supplementary Material

Supplementary material is available online at Open Forum Infectious Diseases (http://OpenForumInfectiousDiseases.oxfordjournals.org/).

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