Monocyte dysfunction is associated with 14-day mortality in cryptococcal meningitis

James Scriven[1,2], Lisa Graham[1], Charlotte Schutz[1], Robert J Wilkinson[1,3,4], Britta Urban[2], Graeme Meintjes[1,3], David Lalloo[2]

1. Clinical Infectious Diseases Research Initiative, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, South Africa. **2** Liverpool School of Tropical Medicine, Liverpool, UK
3. Department of Medicine, Imperial College, London, UK. **3**.MRC National Institute for Medical Research, London, UK

Abstract 836

Abstract

Introduction Cryptococcal meningitis (CM) remains a significant cause of death amongst individuals with HIV-1 infection. Immune correlates of protection are lacking. Animal and *in vitro* studies suggest macrophage and monocyte activation plays a key role in determining outcome from infection. This study examines the relationship between peripheral blood monocyte activation and clinical outcome in patients with HIV-1-associated CM.

Methods Patients with HIV-1-associated CM were recruited from hospitals in Cape Town. Initial anti-fungal therapy was IV amphotericin 1mg/kg and oral fluconazole 800mg per day. Monocyte sub-populations and their activation phenotype were characterized in whole blood at baseline using flow cytometry. Intracellular cytokine responses were assessed following 6-hour stimulation with lipopolysaccharide (LPS) and R848 (TLR7/8 agonist). Comparisons in baseline monocyte activation were made between subjects who survived to 14 days and those who died.

Results 59 subjects were enrolled, median CD4 count was 34x10^6/ml and 14-day mortality was 24%. Compared to survivors (S), non-survivors (NS) had significantly lower expression of HLA-DR, and a trend towards higher expression of CD163, on CD14++CD16+ monocytes at baseline (mean HLA-DR MFI 7272 (S) vs 4197 (NS), p=0.007; mean log10 CD163 MFI 3.92 (S) vs 4.09 (NS), p=0.094 respectively). In addition, following LPS stimulation monocyte cytokine responses were impaired in non-survivors, with significantly fewer monocytes producing TNFα (26% (NS) vs 44% (S), p=0.002). However, there was no difference in monocyte responses following TLR7/8 stimulation.

Conclusions This study suggests impaired monocyte function may be an important factor determining poor outcome in cryptococcal disease. This may reflect overstimulation, and compensatory down-regulation, of inflammatory responses in circulating monocytes. Reversal of this defect may be a potential immune-based therapeutic strategy.

Background

- Despite ART rollout, cryptococcal meningitis (CM) remains a significant cause of mortality amongst HIV-1-infected individuals in sub-Saharan Africa[1].
- Outcome remains poor[2]
- The host immune response is crucial to the pathogenesis of cryptococcosis but immune correlates of poor outcome are not well described in humans[3]

Objective

- To determine whether abnormal monocyte activation and functional responses are associated with poor outcome in HIV-1-associated cryptococcal meningitis

Methods

- Patients with HIV-1-associated CM were recruited from district hospitals in Cape Town
  (Anti-fungal Rx: Amphotericin B 1mg/kg + fluconazole 800mg/d for 14 days)
- Blood samples were taken at enrolment
- Flow cytometry was used to examine peripheral blood monocyte populations and expression of surface activation markers (HLA-DR and CD163; see **figure 1**)
- Monocyte intracellular cytokine responses (TNFα, IL-6, IL-10, IL-12) were assessed following 6-hour stimulation with lipopolysaccharide (TLR4 agonist) or R848 (TLR7/8 agonist)
- Soluble markers of monocyte activation were also measured in serum using commercial ELISA and Luminex assays
- Comparisons were made between subjects who survived to 14 days and those who died: student's T-test was used for normally distributed variables; Mann-Whitney U was used for variables that were not normally distributed (or variables were log transformed prior to T-test); Fisher's exact test was used for categorical variables

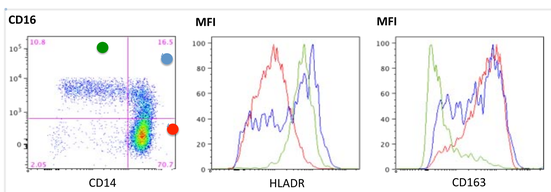


Figure 1.
Monocyte subsets[4] with expression of HLA-DR and CD163.
- Classical (red): CD14++ CD16-:
- Intermediate (blue): CD14++CD16+,
- Non-classical (green): CD14+CD16++.

Results

- 59 patients were recruited; 14-day mortality was 24%
- Compared to survivors, non-survivors had:
  - Significantly reduced circulating monocyte populations **(table 1)**
  - Significantly decreased baseline HLADR expression on CD14++CD16+ monocytes **(table 2, figure 2)**
  - Significantly increased serum concentrations of sCD163, CRP and IL-6 (but not sCD14) **(table 2)**
  - Significantly fewer TNFα positive monocytes following LPS stimulation **(table 2, figure 3)**
- Impaired TNFα production was correlated with higher serum IL-6 concentration **(figure 4)**

Baseline Characteristic	Survivors (n=45)	Non-survivors (n=14)	p value
Age (median, IQR)	36 (31-43)	37 (27-45)	0.936
CD4 (median, IQR)	34 (12-85)	33 (13-45)	0.587
Log10 HIV Viral Load (median, IQR)	5.14 (4.36-5.44)	5.21 (4.69-5.69)	0.351
ART (n, %)	13 (29)	3 (21)	0.738
Serum GXM titre (median, IQR)	6000 (4000, 24000)	8000 (4000, 32000)	0.423
Altered mental state (GCS<15) (n, %)	7 (16)	7 (50)	**0.014**
Log10 Fungal CFUs/ml CSF (median, IQR)	4.40 (3.10-5.45)	5.54 (4.66-6.44)	**0.027**

Table 1. Differences in baseline characteristics between survivors and non-survivors

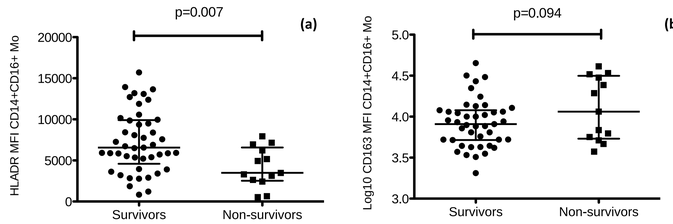


Figure 2.
(a) HLADR expression on "intermediate" monocyte sub-population, significantly lower expression noted among individuals who did not survive to 14 days (median and IQR displayed); **(b)** non-survivors also had a non-significant trend towards increased expression of CD163 on "intermediate" monocytes (median and IQR displayed).

Immune Parameter	Survivors (n=45)	Non-survivors (n=14)	P value
Monocytes (x10^9/L) (mean, 95% CI)	0.35 (0.29-0.41)	0.28 (0.14-0.43)	0.297
Monocytes (%WBC) (mean, 95% CI)	8.0 (6.9-9.0)	5.6 (3.5-7.7)	**0.039**
HLADR MFI CD14++CD16+ Mo (mean, 95% CI)	7272 (6154-8390)	4197 (2340-5653)	**0.007**
CD163 MFI CD14++CD16+ Mo (Log10) (mean, 95% CI)	3.92 (3.83-4.01)	4.09 (3.86-4.33)	0.094
Serum sCD163 (ng/ml) (median, IQR)	1033.5 (765.5-1480.5)	1410 (1196-1856)	**0.017**
Serum sCD14 (ng/ml) (median, IQR)	3278 (2888-3893)	3838 (2931-5183)	0.313
Serum CRP (mg/L) (median, IQR)	36.4 (13-68.3)	84.9 (36.5-115)	**0.009**
Log 10 Serum IL-6 (pg/ml) (mean, 95%CI)	1.40 (1.25-1.55)	1.85 (1.43-2.29)	**0.010**
%TNFα +ve Mo after LPS stimulation (median, IQR)	44.8 (36.4-57.2)	19.2 (9.7-40.7)	**0.004**
%TNFα +ve Mo after R848 stimulation (median, IQR)	79.4 (63.0-83.4)	72.8 (50.8-79.6)	0.191

Table 2. Differences in peripheral blood immune parameters between survivors and non-survivors at baseline

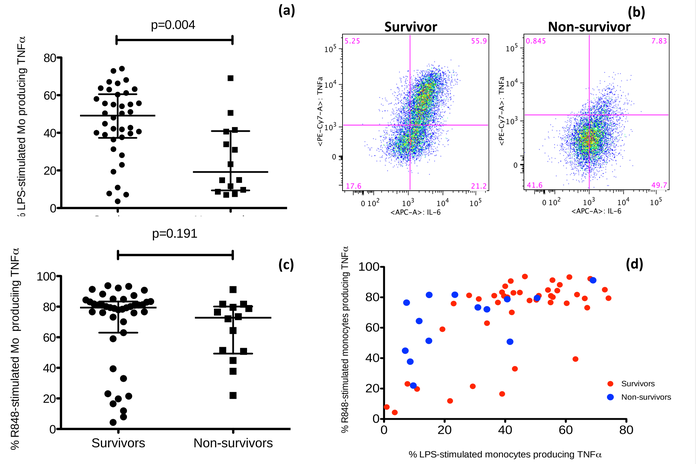


Figure 3.
(a) Baseline intracellular cytokine production of TNFα was significantly impaired in LPS-stimulated monocytes from non-survivors (medians and IQR shown); **(b)** typical monocyte LPS response in a survivor and non-survivor; **(c)** no difference was noted between survivors and non-survivors in TNFα response to R848 (TLR7/8 agonist) (medians and IQR shown); **(d)** relationship between TNFα responses to LPS and R848 stimulation: although LPS TNFα response was impaired in non-survivors, many had a preserved response to R848.

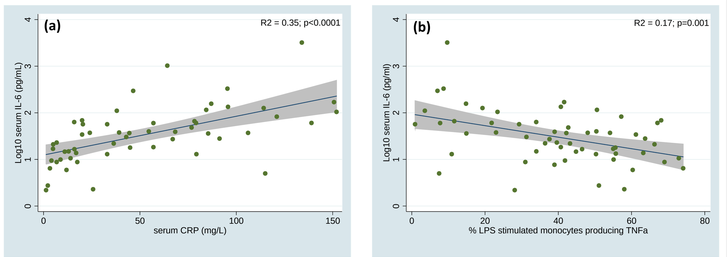


Figure 4.
(a) CRP is positively correlated with log10 serum IL-6 concentration; **(b)** low TNFα production by LPS-stimulated monocytes was correlated with higher baseline serum concentrations of IL-6

Conclusions

- Monocytes from patients who die from cryptococcal meningitis have a less activated phenotype, and impaired production of TNFα following LPS stimulation.
- Given the association with increased soluble markers of monocyte and immune activation, this may reflect overstimulation and a compensatory down-regulation of immune responses, similar to those described in bacterial sepsis[5]
- Reversal of this defect may be a potential immune based therapeutic strategy

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