Characterizing the CSF Cellular Immune Response of Cryptococcus Fungal Burden in HIV+ Ugandans with Cryptococcal Meningitis

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Abstract

Background: Cryptococcal meningitis causes substantial mortality globally, with a one-year mortality rate of 70% in Sub-Saharan Africa. One of the strongest risk factors for mortality includes high cerebrospinal fluid (CSF) fungal burden at baseline. Our understanding of the role of the cellular immune response in CSF in persons with advanced HIV and high CSF cryptococcal fungal burden remains poorly defined. We used flow cytometry to characterize the immune response in CSF and assessed correlations with baseline CSF cryptococcal fungal burden.

Methods:

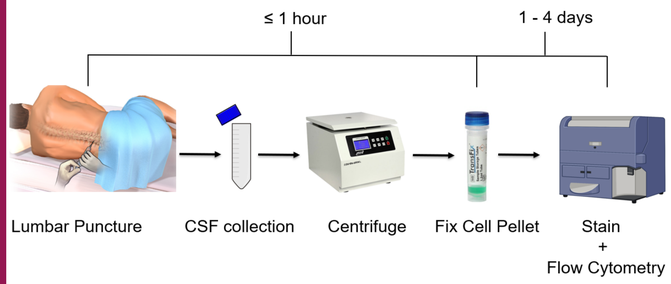


Figure 1. CSF collection workflow. CSF was collected at beside lumbar puncture from 30 patients with cryptococcal meningitis diagnosed by CSF CrAg and quantitative CSF fungal culture. CSF was centrifuged and the cell pellet was fixed within 1 hour of collection. Fixed cell pellets were washed and stained within 1-4 days of collection with CD45, CD3, CD19, CD56, CD14, CD16, CD56, CD134, HLADR, and CD11c antibodies. Cells were analyzed on a Beckman Cytoflex flow cytometer.

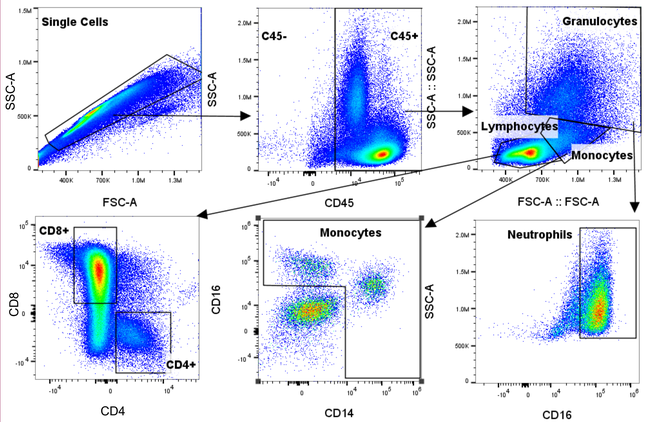


Figure 2. CSF flow cytometry gating. CSF cells showing single cells, CD45+ cells, FSC-SSC plot of major cell subsets: lymphocytes. monocytes, granulocytes. CD8/CD4 plot of CD3+ T cells, CD16/CD14 plot of $CD3^-$ monocytes, and $CD16^-/SSC$ plot of $CD16^+CD14^-$ neutrophils.

Immune Cells Present in the CSF at Baseline

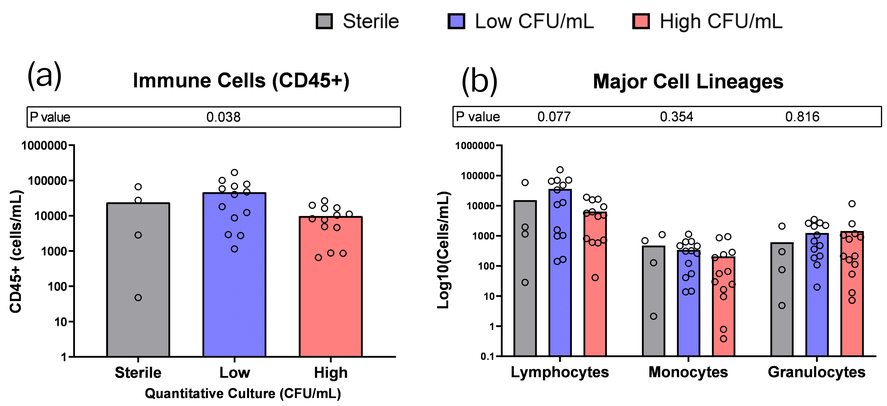


Figure 3. CSF Immune Cell Counts by CSF Fungal Burden. (a) We compared absolute immune CD45+ (pan-leukocyte marker) cells in the CSF to quantitative CSF fungal culture and cellular subsets: (b) Major Cell lineages. P-values were calculated using one-way ANOVA.

T Cell Activation Status

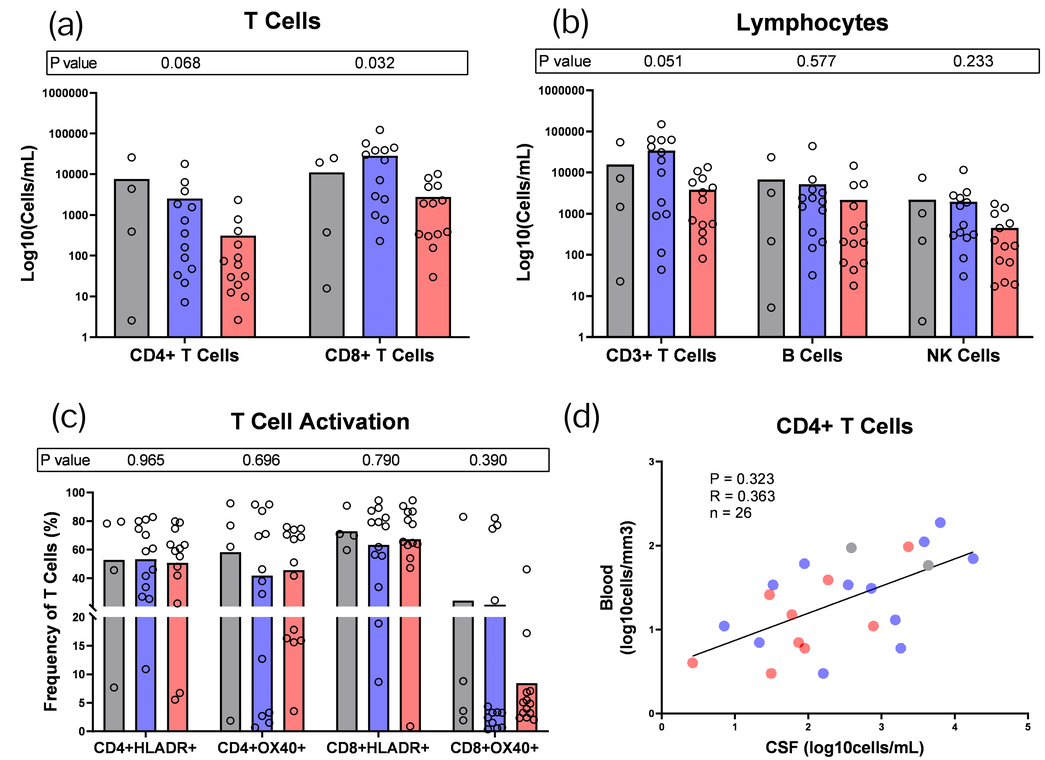


Figure 4. Comparison of T cell activating across CSF fungal burden. (a) We compared the cellular subsets to CSF fungal burden: (a) T cells, (b) Lymphocytes, (c) T Cell Activation, (d) CD4+ T cells in CSF and blood. Absolute cells/mL of CSF were calculated by dividing sample cell counts by the pre-centrifuged CSF volume. Quantitative CSF cultures were categorized as sterile (0 CFU/mL), low (10-17,500 CFU/mL, and high (27,500-195,000 CFU/mL) with low representing the bottom 50th percentile and high as the top 50th percentile of fungal burden. P-values were calculated using one-way ANOVA (a-c) and linear regression (d), respectively.

Clinical Characteristics

Variables	Sterile	Low CFU	High CFU	P Value
Total (N)	4	13	13	-
Age, years	42 (36-55)	42 (37-49)	36 (33-43)	0.325
Sex, male (%)	2 (50%)	7 (50%)	7 (50%)	-
CSF Quant. Culture, CFU/mL	0 (0)	245 (55-3050)	53,000 (35,250-195,000)	-
CSF Opening Pressure, mmH2O	120 (67-142)	138 (82-276)	355 (165-401)	0.030
CSF Closing Pressure, mmH2O	75 (41-105)	68 (53-98)	80 (70-115)	0.570
CSF WBC, wbc/mm3	22 (<5-85)	95 (20-247)	<5 (<5-83)	0.047
CSF Glucose, mmol/L	2.83 (<1.1-4.8)	2.3 (1.3-4.1)	3.6 (2.5-3.8)	0.332
CSF Protein, mg/dL	76 (30.5-165)	100 (70-150.5)	100 (66-114.5)	0.535
CSF Lactate, mmol/L	2.7 (2.5-4.2)	3.6 (2.8-4.9)	3 (2.5-3.7)	0.202
CSF Volume Removed, mL	6.5 (5-7.8)	10 (5.5-21)	21 (13-33)	0.009
Blood Abs CD4	76 (58-94)	32.5 (8-68)	11 (5-32.5)	0.264

Data is shown as N (%) and median (IQR). Continuous variables were compared by one-way ANOVA. IQR= interquartile range.

Conclusion

Our findings suggest:

- Among those with a positive CSF cryptococcal culture, those in the top 50th percentile had the highest opening pressure, CSF volume, and a lower total CD45+ cell concentration than those in the lower 50th percentile
- Higher CSF fungal burden was associated with a weakened CD4 T cell response, with a smaller T cell number and reduced activation status
- CD4+ T cells in the blood were positively correlated with CD4+ T cells in the CSF
- Ongoing sample collection will increase our sample size for future analysis and follow-up studies including paired cytokine analysis to better understand cellular trafficking and homing mechanisms

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