Examining the Effect of Intracellular *C. neoformans* on the Transcriptome of Classically and Alternatively Activated Macrophages

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Introduction

The fungal pathogen and etiological agent of cryptococcosis, *Cryptococcus neoformans (Cn),* is responsible for ~181,000 deaths/year worldwide.

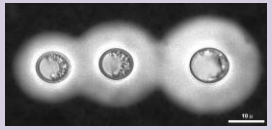


Figure 1. *Cryptococcus neoformans*. India ink-stained images of *C. neoformans* by Dr. Erin McClelland

Macrophages, phagocytic innate immune cells, serve as a first line of defense against *Cn* and strongly influence the outcome of infections. The ability of these cells to destroy ingested *Cn* is dependent on their polarization state.

- The classical (M1) state is highly fungicidal and characterized by inducible nitric oxide synthase (iNos) and pro-inflammatory cytokine expression.
- The alternative (M2) state is largely anti-inflammatory and associated with tissue repair.

These polarization states, which are defined by the differential expression of >1000 genes, serve as temporary phenotypes that provide flexibility of function and can change in response to cytokine signals.
As *Cn* is able to replicate inside macrophages, we hypothesized that it may disrupt the polarization state of host cells to compromise fungicidal activity. We also predicted that these transcriptional changes would differ depending on the initial polarization state.

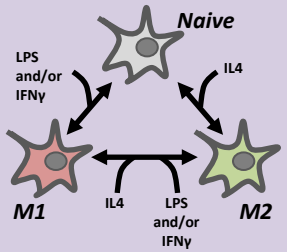
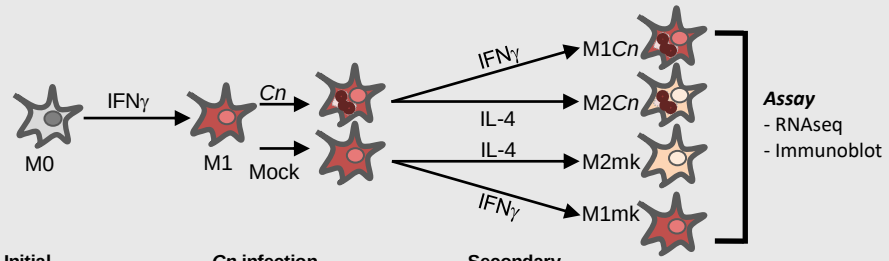


Figure 2. *Macrophage polarization*. The polarization state of macrophages is inherently plastic and they can be polarized and repolarized in response appropriate to stimuli

Methods

M1-polarized RAW 264.7 murine macrophages were infected with H99s *Cn*. Post-infection, cells were maintained in M1 or repolarized to M2 using IFN-γ or IL-4, respectively. Western blot analysis or RNAseq-based transcriptome profiling of host macrophages was performed 24-hours post-infection. RNAseq was followed by gene ontology using DAVID.

Workflow



Initial
- M1-polarize (24 h)

***Cn* infection**
- Infection period is 2 h,
- Extracellular Cn removed

Secondary
- Repolarize (24 h),
- Medium replaced every 6 h

Figure 3. *Schematic representation of the macrophage infection protocol*

M0 vs. M1mk

Gene	FC		q-value
Cxcl9	682.63	UP	0.00626191
Gbp2	369.19	UP	0.00626191
Cd86	356.53	UP	0.0383977
Nos2	150.18	UP	0.00626191
Cxcl10	139.62	UP	0.00626191
Stat1	46.87	UP	0.0334391
IL1b	46.41	UP	0.0161112
Fcgr1	17.61	UP	0.00626191
Ccl5	6.66	UP	0.0370904

M1mk vs. M2mk

Gene	FC		q-value
Arg1	623.88	UP	0.00626191
Atp6v0d2	14.06	UP	0.0350194
IL10	10.08	UP	0.0413593
Cxcl10	18.9	DOWN	0.00626191
Cxcl11	15.77	DOWN	0.0200165
Nos2	12.24	DOWN	0.00626191
Cxcl9	8.84	DOWN	0.00626191
GBP2	5.21	DOWN	0.00626191

Table 1. *RNAseq analysis of M1 and M2 macrophage polarization markers*

Results (1)

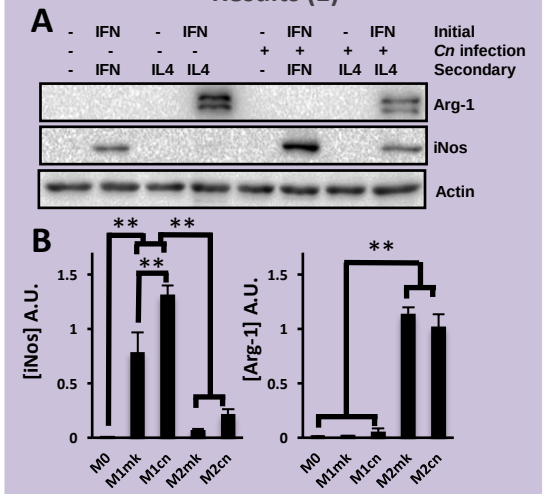


Figure 4. *Intracellular Cn infection affects expression of the M1 marker, iNos at the protein level but not the M2 marker, Arg1.* **(A)** Western blot analysis of protein lysates from cells cultured under the indicated conditions **(B)** Quantification of iNos and Arg-1 levels by densitometry. Data is from six discrete biological repeats. Error is represented as S.E. ** represents $p<0.01$

Results (2)

Concordant up-regulated genes in Cn-infected cells

	M1mk vs. M1cn		*M2mk vs. M2cn*	
Gene	FC	q-value	FC	q-value
Cited1	***14.81***	***6.26E-03***	***20.49***	***1.61E-02***
Hsf3	7.93	2.86E-02	8.04	0.0200165
Jarid2	5.45	2.52E-02	2.88	0.0389813
Tmtc2	5.14	9.61E-03	3.94	0.00626191
Ccl22	5.09	3.50E-02	4.1	0.00626191
Sspn	3.66	4.28E-02	3.21	0.0251778
Wdr89	3.09	3.34E-02	2.43	0.0389813
Bcl2a1a	2.68	4.68E-02	2.95	0.0171929

Concordant down-regulated genes in Cn-infected cells

	M1mk vs. M1cn		*M2mk vs. M2cn*	
Gene	FC	q-value	FC	q-value
Sorl1	37.12	3.90E-02	3.86	0.046023
Dusp6	14.54	1.29E-02	6.72	0.0375289
C5ar1	12.07	6.26E-03	5.45	0.00626191
Ehd2	11.63	6.26E-03	5.08	0.00626191
Grk5	8.83	6.26E-03	4.48	0.0128586
Cd300ld	7.24	6.26E-03	2.7	0.0389813
Zfp146	6.31	1.61E-02	3.97	0.0304857
Endod1	6.21	6.26E-03	2.57	0.027269

Table 2. *Identification of genes affected by intracellular Cn infection in both M1 and M2 polarized macrophages*

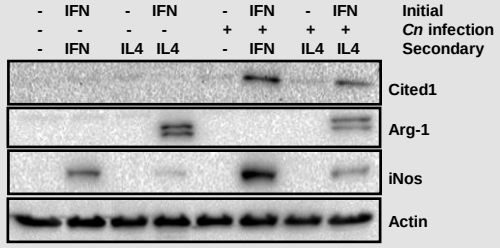


Figure 5. *The transcriptional co-regulator, Cited1 is up-regulated at the protein level in M1 and M2-polarized host macrophages.* Western blot analysis of protein lysates from cells cultured under the indicated conditions. Experiment has been repeated twice.

Discussion

Through a combination of RNAseq and western blot analysis, we found that although *Cn* infection had only modest effects on macrophage polarization state (i.e. small increase in the expression of the M1 marker, iNos), it had broader effects on gene expression in host macrophages. We identified a common set of differentially expressed genes that were affected regardless of initial polarization state. This included upregulation of the anti-apoptotic gene, *Bcl2A1a* and the transcriptional co-regulator, *Cited1*. Future work will investigate how the expression of these genes influences the fate of host macrophages

References

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