Identification of Host-Derived Biomarker Signatures in Cryptococcal Infection

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Background

- Cryptococcal species are capable of producing life-threatening pulmonary disease and meningoencephalitis.
- Clinical differences exist in disease manifestations seen in *Cryptococcus neoformans* (*C. neoformans*) and *Cryptococcus gattii* (*C. gattii*) infection.
- Our understanding of the pathobiology of infection with these two fungal strains is limited, and we don't have great methods for diagnosis and predicting prognosis.
- Challenge studies with other pathogens (e.g., respiratory viruses) have shed light on the host response to infection at the transcriptomic level.[1,2]

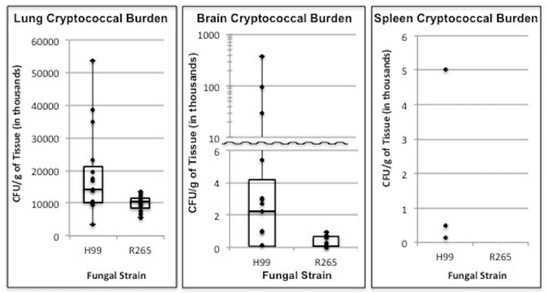
Objective

- Using a *Cryptococcus* challenge model, we attempted to characterize cryptococcal pathogenesis through analysis of host gene expression changes in the infected state.
- We also sought to develop a host gene expression-based disease classifier capable of identifying cryptococcal infection.

Methods

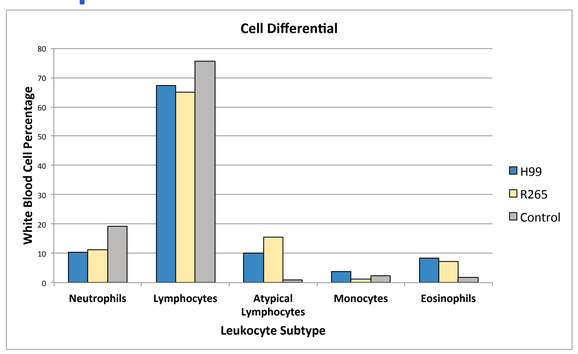
- 45 BALB/cJ female mice at 8 weeks of age were divided equally into 3 cohorts.
- Mice were inoculated intranasally with either $1.0x10^4$ – $1.5x10^4$ *C. neoformans* H99 cells in 25 µL PBS, *C. gattii* R265 cells in 25 µL PBS, or sham control.
- After 14 days, mice were sacrificed for examination of fungal tissue burden and collection of whole blood for microarray analysis.

Fungal Tissue Burden



Mice infected with *C. neoformans* H99 demonstrated more severe fungal burden in lung, brain, and splenic tissues than those infected with *C. gattii* R265.

Complete Blood Count Differentials



Peripheral white blood cell counts revealed differential responses to infection. Both *C. neoformans* H99 and *C. gattii* R265 infections induced peripheral eosinophilia and atypical lymphocytosis.

Cryptococcal Disease Signature

- A Lasso logistic regression model was used to develop a disease classifier. A 4-fold cross validation was used to assess model accuracy.
- A 28-probe classifier, consisting of 27 unique genes, classified controls from infected mice with 100% accuracy.
- No classifier could be generated from this model that separated *C. neoformans* and *C. gattii* infection.

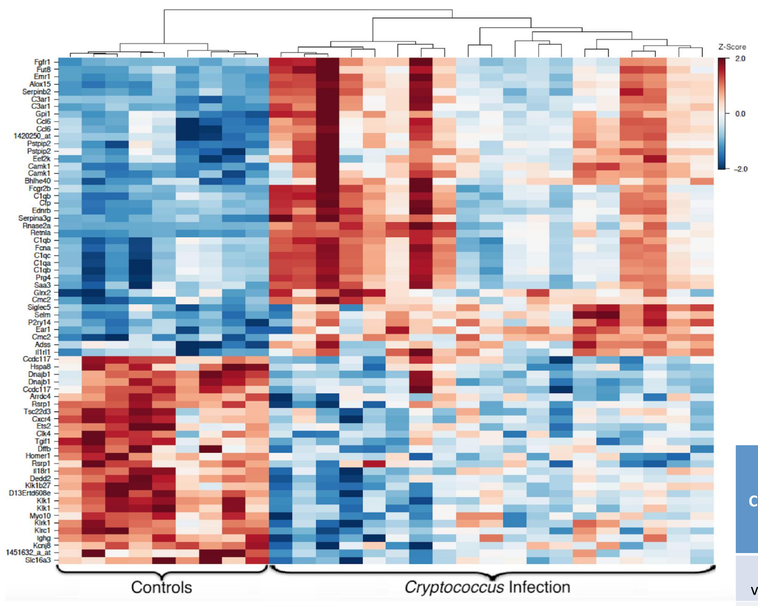
Conclusions

- For the first time, we have examined the host response to cryptococcal disease through the lens of gene expression in circulating white blood cells.
- *C. neoformans* generates a more powerful host transcriptomic response, with a greater degree of complement activation and T_H2-skewing.
- Test like these may provide diagnostic and prognostic information, with a classifier capable of distinguishing *Cryptococcus* infection with 100% accuracy.

Limitations

- Only one time point and one primary site of infection (pulmonary) were studied.
- Additional mice strains and multiple cryptococcal isolates need to be study to more completely define transcriptomic changes in cryptococcal infection.
- The classifier was developed against uninfected controls, whereas a clinically useful classifier should be validated in populations with similar syndromic presentations to determine its ability to distinguish fungal from bacterial and viral infection.

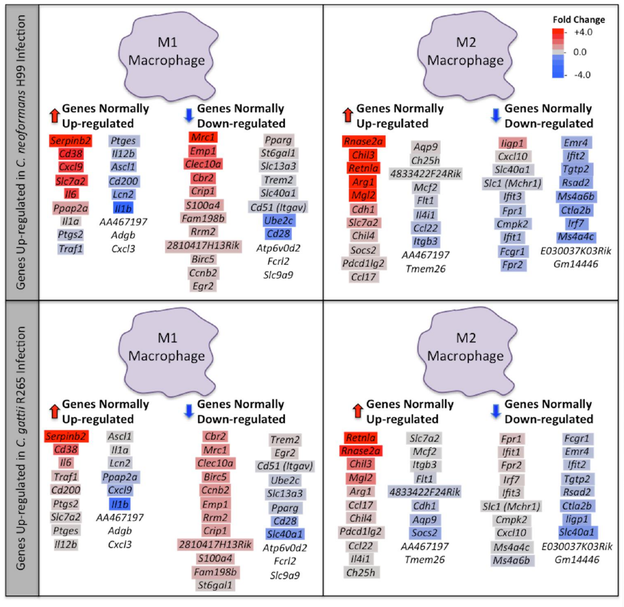
Host Gene Expression Changes



Heat map containing 68 genes differentially expressed between healthy and infected mice.

- Mice infected with the two *Cryptococcus* strains exhibit a powerful transcriptomic response to infection with broadly conserved components.
- Notable differences include a greater degree of complement activation and more T_H2-skewing (as seen through M2 macrophage activation) in *C. neoformans* H99 infection.

Comparison	Significant Genes (p<0.05)	Genes with at least 2-fold Change	Genes with FDR-corrected p<0.05 and at least 2-fold Change
H99 vs. Controls	8062	461	67
R265 vs. Controls	1584	81	10
(H99 + R265) vs. Controls	3426	87	25



Host differentially expressed genes and their role in M1 and M2 macrophage activation.[3]

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References

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