Immunological relevance of the mammalian receptor G2A in host response to *Aspergillus fumigatus* pulmonary infection

Breanne Steffan[1], Dante Calise[1], Nancy P. Keller[1,2]

[1]Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, Madison, WI, USA
[2]Department of Bacteriology, University of Wisconsin-Madison, Madison, WI, USA
E-mail address: bsteffan@wisc.edu

Abstract

Purpose

Aspergillus fumigatus is the main causative infectious agent of invasive aspergillosis, a fungal infection with up to 90% mortality[1]. Understanding the immunological mechanism behind the initiation and establishment of fungal growth within the lung is still widely unknown. An area of increasing interest is in the role of oxygenated fatty acid (oxylipin) signaling in disease pathogenesis. G2A is a vertebrate GPCR with known oxylipin ligands including 9-HODE and 13-HODE[2]. Absence of G2A in inflammatory diseases, including infections, results in altered immune cell recruitment, polarization, and cytokine production[2,3]. We hypothesize that G2A plays a critical role in the establishment of infection by *A. fumigatus* through altered inflammatory processes resulting in more critical disease outcomes.

Methods

Immunocompetent G2A$^{+/+}$ and G2A$^{-/-}$ mice were infected with *A. fumigatus* (CEA10) intranasally and monitored for ten days for survival. An early timepoint (D1 pi) was chosen to assess changes to immune cell egress (flow cytometry) to the lungs, fungal burden, and cytokine/chemokine production (ELISA) from bronchoalveolar lavage.

Results

G2A$^{-/-}$ mice were less likely to succumb to death upon infection in comparison to G2A$^{+/+}$ mice. Both groups showed increased neutrophilia at D1 pi, with a trend toward more neutrophils in the G2A$^{-/-}$ animals. However, G2A$^{-/-}$ mice had a significantly lower percent population of CD11b^{+}CD11c^{-} macrophages in comparison to G2A$^{+/+}$ mice. IL-6 production is significantly higher in G2A$^{-/-}$ mice at D1 pi when compared to G2A$^{+/+}$ mice.

Conclusions

Immune responses to *A. fumigatus* are more effective at preventing infection related mortality when G2A is absent in comparison to G2A$^{+/+}$ animals. Changes in IL-6 production and immune cell egress to the lungs in G2A$^{-/-}$ mice is likely critical to disease development and will be explored further in future studies.

References

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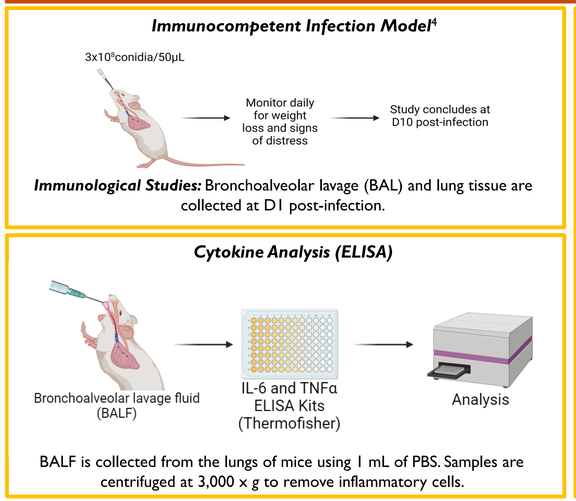
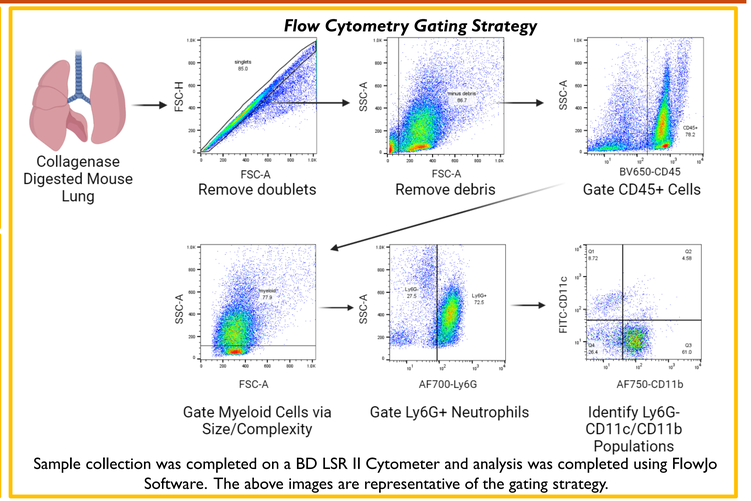
Methods





Results

I. G2A KO mice infected with *A. fumigatus* have greater survival in comparison to WT mice.

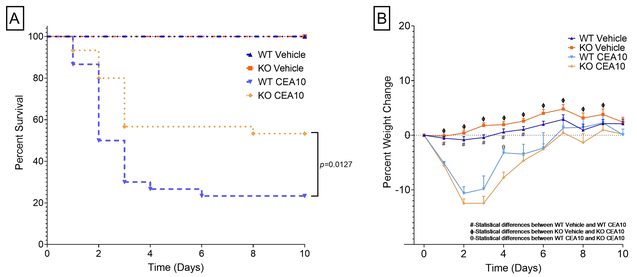


Figure I. G2A KO infected mice exhibit greater survival in comparison to WT mice. Survival data (A) were pooled from three independent studies. *P* values were calculated by Cox proportional hazard regression analysis. Percent weight change over time (B) is shown as average ± SEM. Statistical analysis was calculated using Student's *t* test with Welch's corrections and statistical differences are shown between WT Vehicle and WT CEA10 (#), KO Vehicle and KO CEA10 (ϕ), and WT CEA10 and KO CEA10 (θ).

II. G2A KO mice have significantly less CD11b+CD11c- cells in comparison to G2A WT mice following *A. fumigatus* infection.

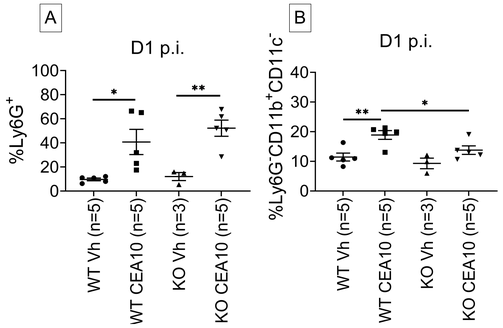


Figure II. CD11b+CD11c- percent cells are significantly decreased in G2A KO mice in comparison to WT mice upon fungal infection, though there is still an overall increase in neutrophils in both groups. Using the abovementioned gating strategy, the populations of myeloid cells from collagenase digested lungs were assessed for population differences using surface cell markers. Each point represents a specific mouse and the overall data shows percent cells ± SEM. Statistical analysis was completed using the Student's *t* test with Welch's correction. Statistical significance is represented by *p<0.05. **p<0.01.

Results (cont.)

III. IL-6 production is significantly increased in G2A KO mice in comparison to WT mice

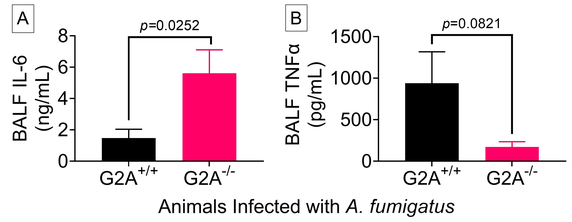


Figure III. IL-6 is significantly increased in G2A KO mice and TNFα is reduced in comparison to G2A WT mice. IL-6 and TNFα protein concentration from the BALF of mice infected with *A. fumigatus are shown as the average* ± SEM. Vehicle controls did not have detectable cytokine levels. Statistical analysis was completed using the Student's *t* test with Welch's correction. Statistical significance is p<0.05.

Conclusions/Future Directions

- ❖G2A KO animals survive infections with *A. fumigatus* better than WT animals.
- ❖G2A KO animals have the greatest percent weight loss at D4 post-infection in comparison to WT infected and vehicle controls.
- ❖Both G2A WT and KO mice have significant increases in Ly6G+ neutrophils
- ❖*A. fumigatus* infected G2A KO mice have significantly fewer percent CD11b+CD11c- myeloid cells than the G2A WT infected mice.
- ❖G2A KO mice have significantly greater IL-6 in the lung lumen.
- ❖TNFα production is reduced in G2A KO infected animals in comparison to WT though not significantly.

Future directions: Infect IL-6 antibody depleted G2A WT and KO animals to determine if survival of G2A KO animals is dependent on IL-6 activity. Because G2A is a known receptor for oxylipins, we aim to discover if host oxylipins are altered during infection resulting in a modulated immune response. Alternatively, we seek to discover whether fungal derived oxylipins can act as immune modulators through G2A[5].

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