Establishment of a post-influenza aspergillosis model in corticosteroid-immunosuppressed mice

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Background

Post-influenza aspergillosis (PIA) is a feared complication in patients with severe influenza, especially those receiving corticosteroids. However, validated murine models of PIA in a background of corticosteroid immunosuppression are lacking, compounding efforts to better characterize the immunopathology and treatment of this emerging entity. Therefore, we established a novel PIA mouse model that seeks to recapitulate realistic clinical timelines and allows us to compare the outcomes of PIA in mice with and without corticosteroid immunosuppression.

Methods

PIA infection model: 8-week-old female BALB/c mice were infected with 1.5% or 7.5% of the 90% lethal dose (LD_{90}) of a mouse-adapted influenza A/Hong Kong/1968 (H3N2) strain (influenza A virus, IAV), delivered by aerosolization. Aerosolized saline was used as a control. Mice then received two intraperitoneal injections of 10 mg cortisone acetate (CA) or mock injections on days 5 and 8 after influenza infection. On day 9, mice were intranasally challenged with 50,000 *Aspergillus fumigatus* AF-293 conidia or mock-infected with saline. Survival and infection severity were monitored until day 16. Infection severity was scored using the viral pneumonia score (VPS)[1] and the modified murine sepsis score (MSS, 0 = healthy to 3 = moribund)[2]. Animals that died prior to the day of assessment received an MSS of 4. Fungal burden was determined in lung tissue homogenates on day 16 or upon death using an 18S qPCR assay[3]. Experimental procedures and end points are summarized in Figure 1.

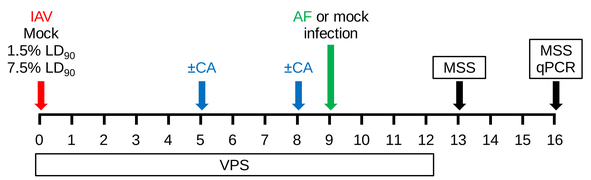


Figure 1: Overview of experimental procedures and readouts for model establishment.

Prophylactic and therapeutic interventions: For therapeutic studies, mice were infected and immunosuppressed as described above. Mice then received daily tail vein injections of 5 mg/kg liposomal amphotericin B (LAmB) or 5% glucose (mock treatment) on days 9 through 13 (0-4 days after AF infection). Alternatively, mice received a 30-minute nebulization of 10 mL of Pam2-ODN (4 µM/1 µM) or PBS at 10L/min on day 8.

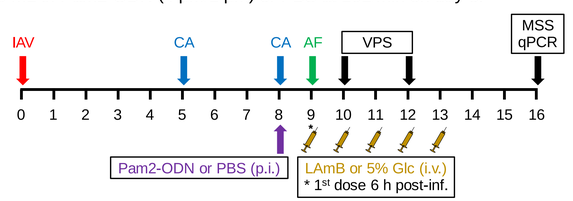
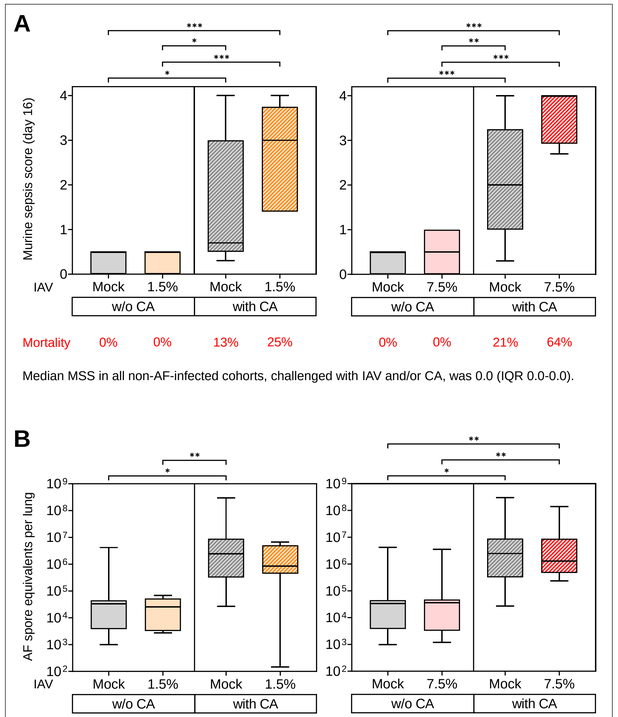


Figure 2: Overview of experimental procedures and readouts for therapeutic studies.

Results



Median MSS in all non-AF-infected cohorts, challenged with IAV and/or CA, was 0.0 (IQR 0.0-0.0).

Figure 3: Cortisone acetate treatment predisposes mice to increased morbidity and mortality due to PIA.
To optimize the PIA model, mice were challenged with IAV (mock infection, 1.5% LD_{90}, or 7.5% LD_{90}) and treated or not with CA, as described in the Methods section. Mice were subsequently infected with AF and monitored until day 16 (7 days after AF infection). **(A)** Distributions of morbidity scores (MSS) on day 16 depending on IAV inoculum and CA treatment. N = 14-16 per group from 3 independent experiments. **(B)** Pulmonary fungal burden on day 16 or upon earlier natural death. N = 10 per group from 2 independent experiments. **(A-B)** Boxes indicate medians and inter-quartile ranges (IQR). Whiskers indicate the spread (min/max). Kruskal-Wallis test with Dunn's post test. * p<0.05, ** p<0.01, *** p<0.001.

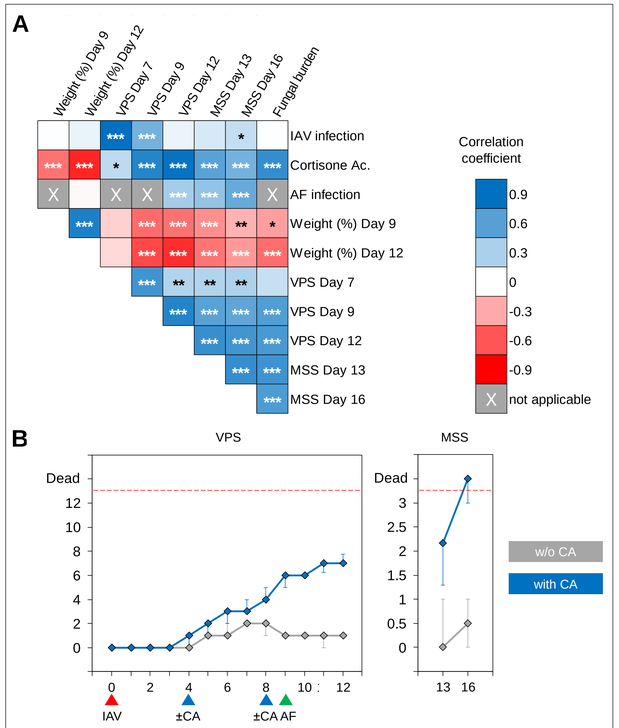


Figure 4: Cortisone acetate is the key predictor of adverse outcomes in our PIA infection model.
(A) Heat map comparing correlation coefficients between experimental challenges and outcomes (weight, VPS, MSS, and fungal burden). Spearman's rank correlation and rank-biserial correlation were used for comparisons of two continuous variables and comparisons of continuous and dichotomous variables (e.g., CA treatment yes/no), respectively. N = 14-15 per group from 3 independent experiments. * p<0.05, ** p<0.01, *** p<0.001. **(B)** Kinetics of VPS and MSS morbidity/mortality scores in IAV- (7.5% LD_{90}) and AF-infected mice depending on CA treatment. Median and IQR are shown.

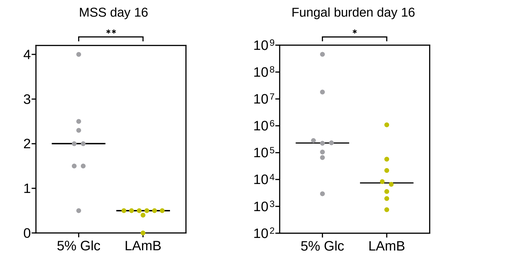


Figure 5: Antifungal treatment improves morbidity, mortality, and fungal clearance in CA-immunosuppressed mice with PIA.
Mice were infected with IAV (7.5% LD_{90}) and treated with CA, as described in the Methods section. On days 9 through 13 (0-4 days after AF infection), mice received intravenous injections of LAmB or 5% glucose (solvent of LAmB, mock treatment). MSS morbidity/mortality scores and fungal burden on day 16 are shown. N = 8 per treatment. Black lines indicate medians. Mann-Whitney U test. * p < 0.05, ** p < 0.01.

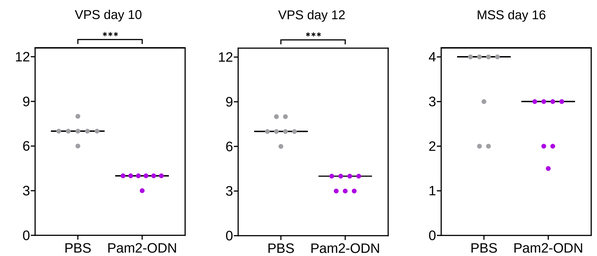


Figure 6: Prophylactic treatment of CA-immunosuppressed, IAV-infected mice with pattern recognition receptor (PRR) agonists improves influenza- and PIA-associated morbidity and mortality.
Mice were infected with IAV (7.5% LD_{90}) and treated with CA, as described in the Methods section. On day 8 (1 day prior to AF infection), mice were nebulized with PBS (mock treatment) or the PRR agonists Pam2 and ODN. VPS and MSS morbidity/mortality scores on representative days are shown. N = 7 per treatment. Black lines indicate medians. Mann-Whitney U test. *** p < 0.001.

Conclusions and Outlook

- **We have established a unique murine PIA model that allows us to compare the course and pathophysiology of IAPA in mice with and without CA immunosuppression.**
- **Our results underscore that corticosteroids are a major driver of PIA-associated morbidity and mortality.**
- **We further validated our PIA model for therapeutic studies by demonstrating protective activity of conventional antifungal therapy (liposomal amphotericin B).**
- **Prophylactic immunomodulatory treatment of CA-immunosuppressed IAV-infected mice with PRR agonists improved influenza- and PIA-associated morbidity and mortality.**
- **In the future, we will employ this novel *in-vivo* platform to study the impact of various antifungal, antiviral, and immunotherapeutic interventions on the natural history and immune pathogenesis of PIA.**

References: 1) Rouxel et al., 2016, Plos One; 2) Mai et al., 2018, Intensive Care Med Exp.; 3) Ibrahim et al., 2005, Antimicrob Agents Chemother.

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