Effect of IL-10 blockade on the circadian gating of lung inflammation

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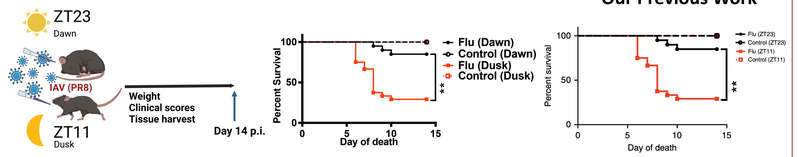
Background

- **The circadian clock controls several aspects of host-pathogen interaction.**
- **Circadian rhythms provide a time-of-day specific protection from mortality in Influenza A Virus (IAV) PR/8 infection that is lost in clock-disrupted mice.**
- **Circadian protection from IAV is independent of viral burden and associated with increased inflammation.[1]**

Our Previous Work[2]



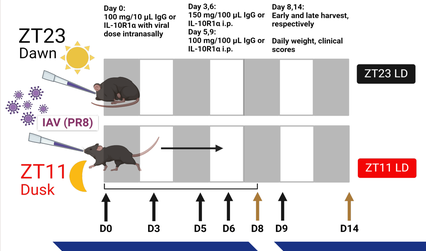
Lights "on": Zeitgeber time "0" (ZT0). ZT23 marks time just before onset of rest (Dawn). ZT11 marks time just before onset of activity (Dusk) since mice are nocturnal species.

- **We have seen that key characteristics of ZT23 subset are increased levels of IL-10 and NK cells, which resulted in elevated survival rates for the dawn-infected mice against the acute viral infection.**
- **IL-10 blockade using IL-10R1a antibody was conducted to determine whether the presence of IL-10 was a main driving force behind the circadian protection of the ZT23 group.[3]**

Aim

- **To determine the role of IL-10 in mediating circadian gating of lung inflammation in IAV, including lung repair and regeneration**

Experimental Design/Methods



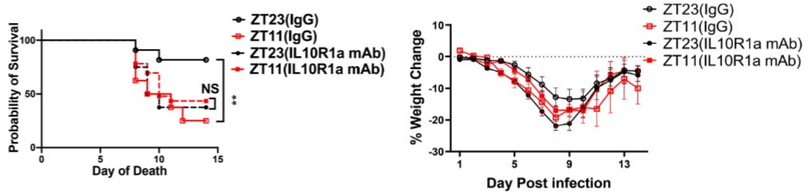
- **Mice: 10-20 weeks old C57bl6/J mice (≈ equal male: female ratio)**
- **Specially designed circadian cabinets to acclimatize mice to reverse light cycling (ZT23: Dawn, ZT11: Dusk)**
- **Locomotor activity via IR sensors (Actimetrics)**

Infection (H1N1 PR/8) → **Monitoring weight loss and clinical scores** → **Histology + D14 KRT5, LAMP-3 IF Staining**

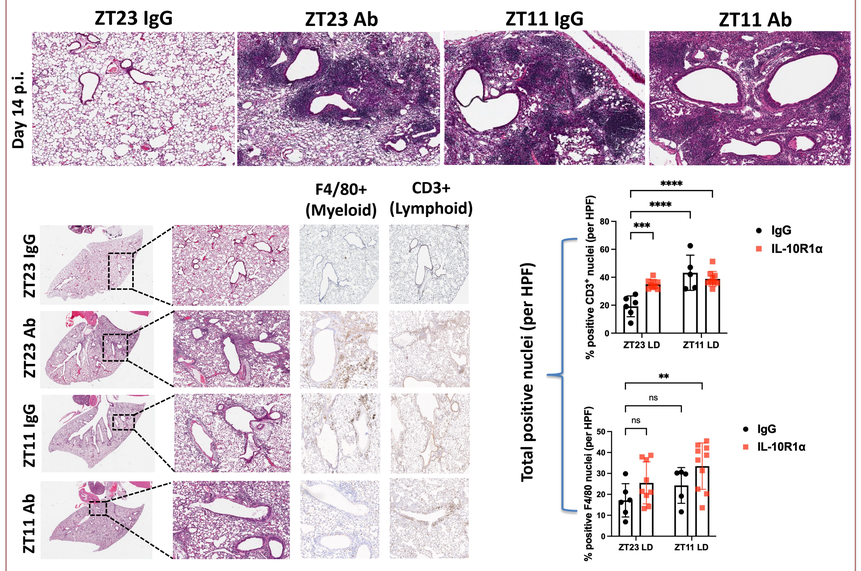
Immunofluorescence Co-Staining Protocol

Deparaffinization/Hydration	2º Antibody Addition (Alexa-Fluor 594 1:250)
Heat Based Antigen Retrieval	Quenching Autofluorescence + Blocking
Quenching Autofluorescence	1º Antibody Addition (LAMP-3 1:500)
Blocking (5% Normal Donkey Serum)	2º Antibody Addition (Alexa-Fluor 647 1:400)
1º Antibody Addition (KRT5 1:50)	DAPI + Mounting + Imaging

Results

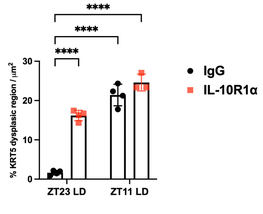


IL-10 blockade due to IL-10R1a resulted in significantly worsened survival in ZT23 group as well as more prominent weight loss. H&E images below also show significantly worsened pathology for ZT23 Ab (IL-10R1a) group compared to IgG.

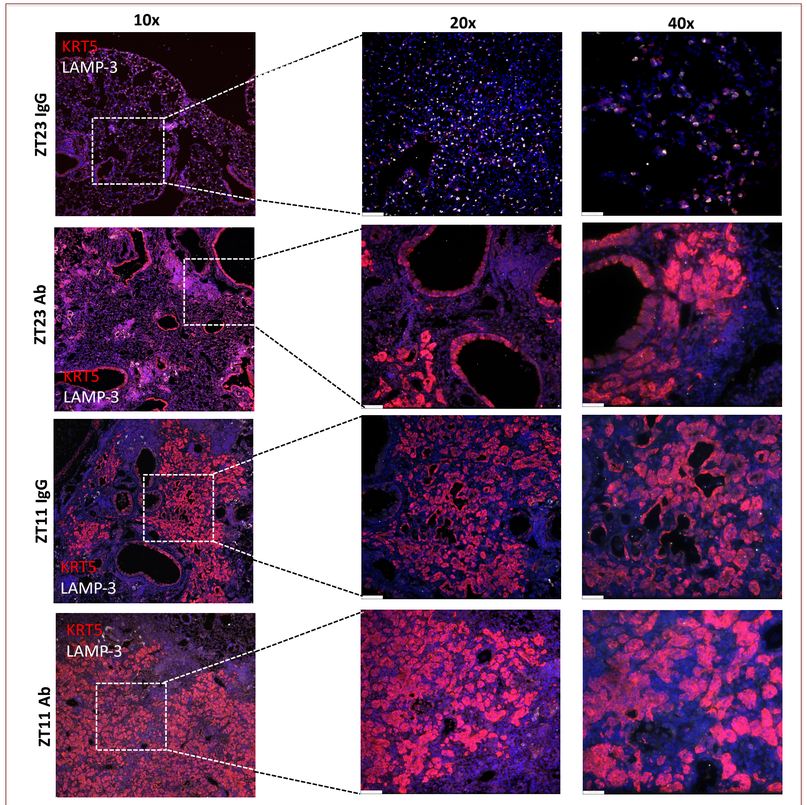


Immunohistochemistry for CD3, marker for lymphocytes, and F4/80, marker for the myeloid population, were conducted. ZT23 IgG control group exhibited significantly less lymphocytes compared to other groups, suggesting that increased lymphocytes in the infected region is the driving force behind severe inflammation. F4/80 is a pan-marker for dendritic cells, macrophages, monocytes, and more lineages. Therefore, F4/80 is not able to detect specific cell types to prove causality.

- **Upon severe lung injuries (due to Covid-19 or H1N1 PR/8), basal stem cells produce KRT5 in damaged alveolar regions instead of normal repair from AT2 cell proliferation**
- **This causes KRT5 dysplasia which is not a normal, but an immediate repair from severe lung damages.**



HPF = High-Power Field
*D8 p.i.



Staining for KRT5 and LAMP-3 on D14 samples using Leica Fluorescence Microscope

Conclusion/Future Directions

- **Conclusion: Blockade of IL-10 signaling abrogates the time-of-day specific protection from IAV.**
- **Future Directions: How do other cytokine or chemokine (e.g. Interferon-gamma) levels change due to IL-10 blockade? How does this relate to the magnitude of T-cell activation?**
- **Does IL-10 play a pivotal role in basal stem-cell induced response of producing KRT5 in alveolar regions? Would IL-10 blockade group still produce KRT5 for immediate repair?**

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