The pathogenesis of and immune response to SARS-CoV-2 oral challenge in K18-hACE2 mice

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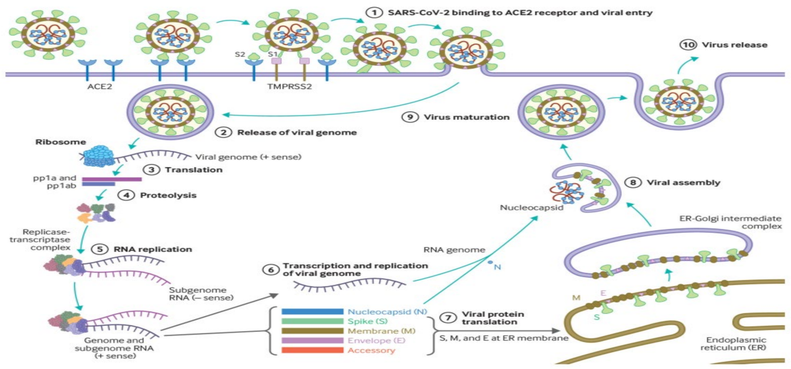
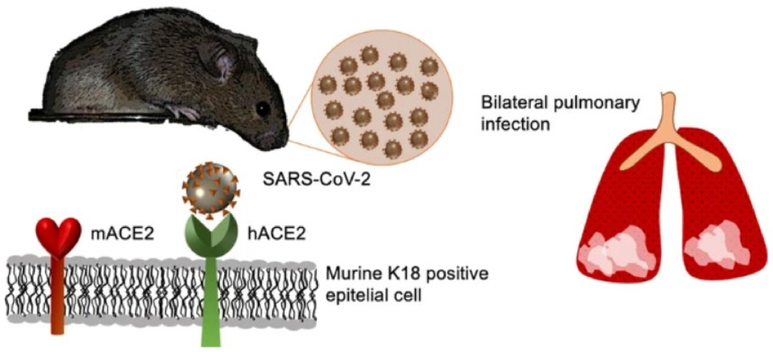
Background

- COVID-19 is an infectious disease caused by the SARS-CoV-2 virus and as of this year, has reached a worldwide mortality of 6.38 million citizens.
- It binds to the cell surface protein ACE2 through the receptor binding domain of its spike protein to infect human cells.



- K18-hACE2 transgenic mice are essential because the human keratin 18 promoter directs expression to airway epithelia, where replication and infection typically begin.



- The primary mode of transmission is inhalation via infected respiratory droplets.
- A secondary mode of transmission to consider is oral via environmental fomites.

Objectives

- Do K18-hACE2 mice become infected with SARS-CoV-2 when orally challenged?
  - Survival rates
  - Clinical signs
- What type of immune response develops?
  - Viral loads
  - Histology

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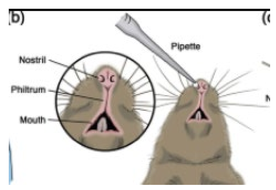
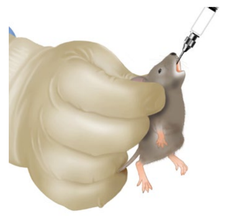
Experimental Methods

Inoculation

- Intranasal:
  - anesthetize with isoflurane.
  - administer 2 x 15 µL droplets of SARS-CoV-2 (1/naris).
    - keep supine until droplets have been inhaled.



- Oral gavage:
  - scruff mice with non-dominant hand.
  - administer assigned concentration in a 100 µL volume with a 1cc syringe fitted with a disposable plastic feeding tube.



Post-inoculation

- Endpoints
  - Intranasal = humane or 14 days post-infection.
  - Oral gavage = humane, 6 days, or 14 days post-infection.

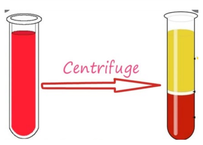
Tissue Collection

Half of both the brain and lungs.

Intestines and half of both the brain and lungs.

Histology

Serum Collection



Conclusions

- Mice that were challenged orally showed some fatalities, weight loss, and lung histopathology similar to mice challenged intranasally.
- These findings indicate that oral inoculation should be looked at as a secondary mode of transmission.
- Additionally, all fatalities represented in both trials were GM1+SFB and GM1 mice.
- This finding indicates that gut microbiota should be investigated more thoroughly to determine susceptibility to SARS-CoV-2.

Future Directions

- Evaluate cytokines for trials 1 and 2.
- Evaluate histology for trial 2.
- Conduct more and larger orally challenged studies.

Results

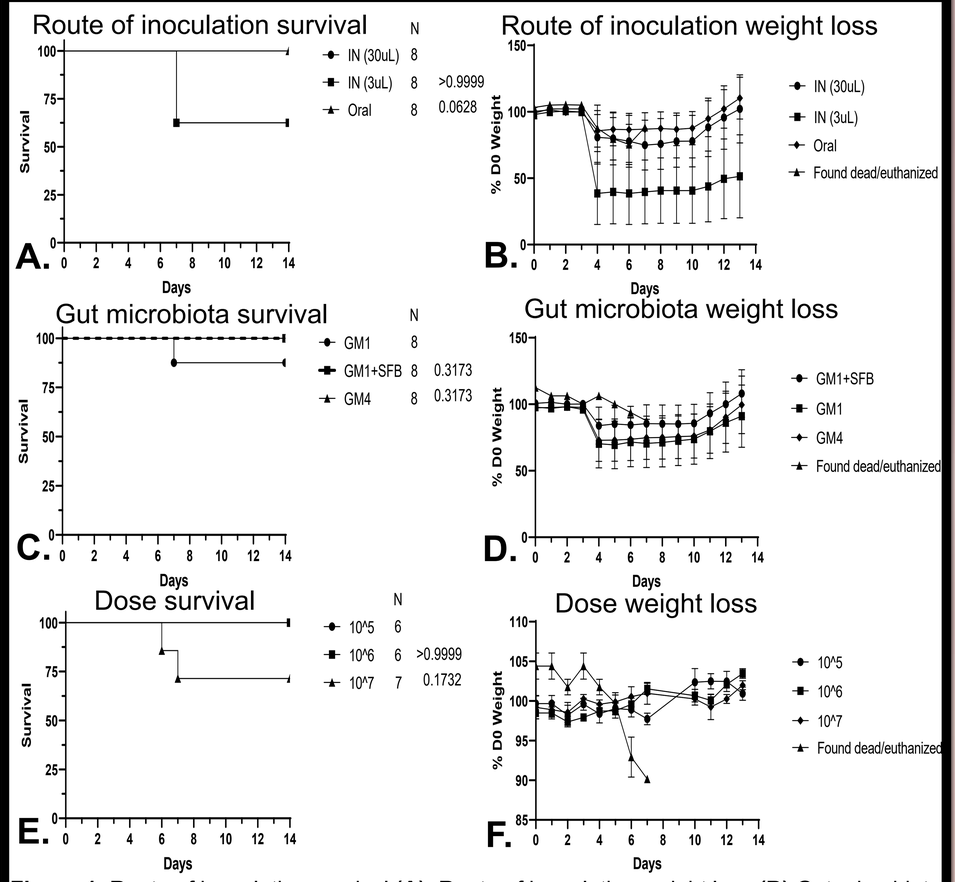


Figure 1. Route of inoculation survival **(A)**, Route of inoculation weight loss **(B)** Gut microbiota survival **(C)**, Gut microbiota weight loss **(D)**, Dose survival **(E),** and Dose weight loss. **A.** 6 GM1+SFB mice inoculated intranasally began showing clinical signs 5-6 dpi and were either found dead or had to be euthanized 7 dpi. **B.** 6 GM1+SFB mice inoculated intranasally lost between 2.5-5 grams of their starting body weight at their humane endpoint. **C.** 1 GM1 mouse inoculated orally began showing clinical signs 5 dpi and had to be euthanized 7 dpi. **D.** 1 GM1 mouse inoculated orally lost 3.5 grams of her starting body weight at her humane endpoint. **E.** 2 GM1 mice inoculated orally with very high doses began showing clinical signs 6 dpi and had to be euthanized 6-7 dpi. **F.** 2 GM1 mice inoculated orally with very high doses lost 3 grams of their starting body weight at their humane endpoint.

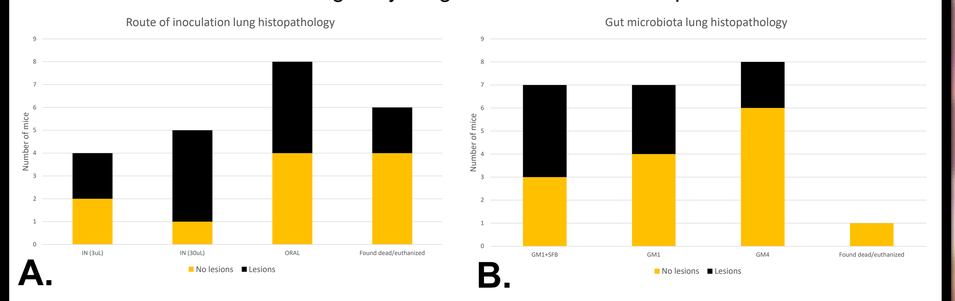


Figure 2. Route of inoculation lung histopathology **(A)** and Gut microbiota lung histopathology **(B)**. **A.** 2 of the 6 GM1+SFB mice that were inoculated intranasally showed pneumonia on histology. **B.** 1 GM1 mouse that was inoculated orally did not show pneumonia on histology.

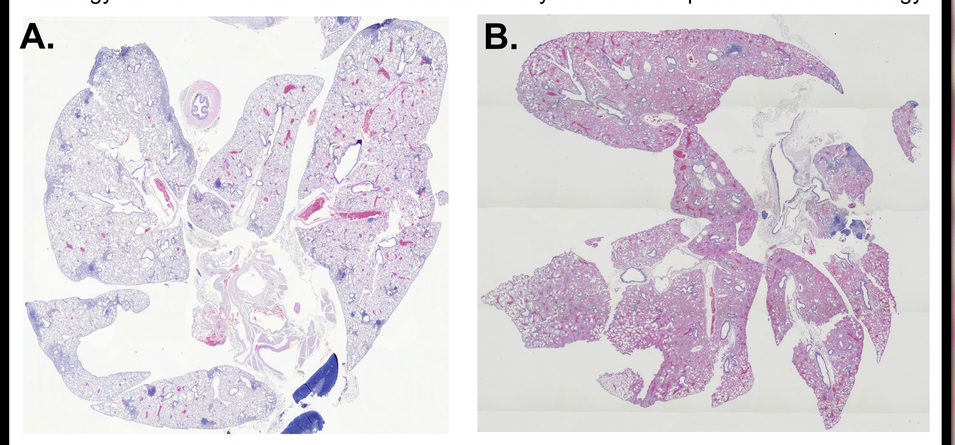


Figure 3. H&E stains of lung tissue taken at 4X magnification. **A.** GM1+SFB mouse inoculated intranasally with 30 µL showing multifocal, lymphocytic interstitial pneumonia. **B.** GM1+SFB mouse inoculated orally showing multifocal, lymphocytic interstitial pneumonia.