Characterization of disease progression in a mouse model of COVID-19

Nikki Seilhamer, James Chung, Rachel Olson, James Amos-Landgraf, Craig Franklin

Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia, Missouri

Veterinary Research Scholars Program
University of Missouri

MMRRC
Mutant Mouse Resource & Research Centers

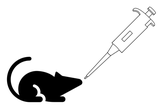
Background

- SARS-CoV-2, the agent of COVID-19, remains a threat to public health
- Severe clinical cases can result in bilateral pneumonia and subsequent ARDS, driven by a proinflammatory cytokine storm
- The B6.Cg-Tg(K18-ACE2)2Prlmn/J mouse expressing human angiotensin-converting enzyme 2 (ACE2) is used to study SARS-CoV-2 pathogenesis
- Initial studies have shown that mice develop interstitial pneumonia during acute disease; however, the full scope of disease is not yet understood

Objective: Characterize disease progression in SARS-CoV-2 infected K18-hACE2 mice over an 8-week period, with a focus on lung and brain cytokines and histopathologic changes.

Methods

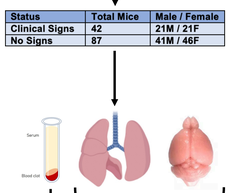
A



1. Intranasal inoculation with 30 mL of SARS-CoV-2 stock solution diluted to 10^5 $TCID_{50}$

Status	Total Mice	Male / Female
Clinical Signs	42	21M / 21F
No Signs	87	41M / 46F

2. Mice euthanized one-week post-inoculation to study acute disease

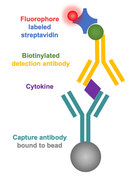


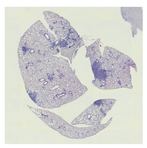
3. Collection of serum, lungs and brain at necropsy.

TRIzol Formalin

4. Half of tissues were homogenized in TRIzol and half were preserved in formalin

5a. Multiplex cytokine assay





5b. Histological analysis of lesion severity in lungs and brain

B

BAFF	IFN gamma	IL-6	IL-17A (CTLA-8)	IL-31	MCP-3 (CCL7)
Betacellulin (BTC)	IL-1 alpha	IL-7	IL-18	IL-33	MIP-1 alpha (CCL3)
ENA-78 (CXCL5)	IL-1 beta	IL-7R alpha	IL-19	IL-33R (ST2)	MIP-1 beta (CCL4)
Eotaxin (CCL11)	IL-2	IL-9	IL-22	IP-10 (CXCL10)	MIP-2 alpha (CXCL2)
G-CSF (CSF-3)	IL-2R	IL-10	IL-23	LIF	RANKL
GM-CSF	IL-3	IL-12p70	IL-25 (IL-17E)	Leptin	RANTES (CCL5)
GRO alpha (CXCL1)	IL-4	IL-13	IL-27	M-CSF	TNF alpha
IFN alpha	IL-5	IL-15	IL-28	MCP-1 (CCL2)	VEGF-A

C

Sample collection (weeks post-inoculation)	Total Mice	Male / Female
3 weeks	14	8M / 6F
4 weeks	13	7M / 6F
5 weeks	16	8M / 8F
6 weeks	11	6M / 5F
7 weeks	14	7M / 7F
8 weeks	10	5M / 5F

Figure 1. A) Schematic of methods for characterization of acute disease. B) Table illustrating cytokines that were tested in the 48-plex assay. C) Table illustrating mice used to study ongoing disease and resolution. Cohorts of mice were euthanized weekly for collection of brains and lungs used for histologic analysis.

Transition from lymphohistiocytic interstitial pneumonia to lymphoplamacytic aggregates

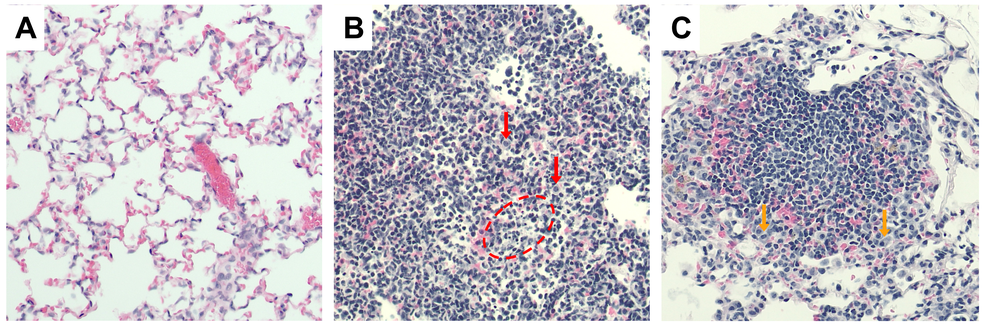


Figure 2. Representative images of mouse lung histology, stained with H&E and taken at 20x magnification. A) Normal mouse lung. B) Lung lesion from a mouse euthanized 6-days post-inoculation. Red arrows indicate alveolar macrophages, and the red dashed circle denotes an area with apoptotic debris. Typically, acute disease (6-8 days post-inoculation) was characterized by a multifocal mild to moderate lymphohistiocytic interstitial pneumonia. C) Lung from mouse euthanized 8-weeks post-inoculation. Orange arrows indicate plasma cells. Lung lesions at 8-weeks were primarily mild multifocal lymphoplasmacytic aggregates which was suggestive of ongoing disease resolution

Meningoencephalitis during acute disease

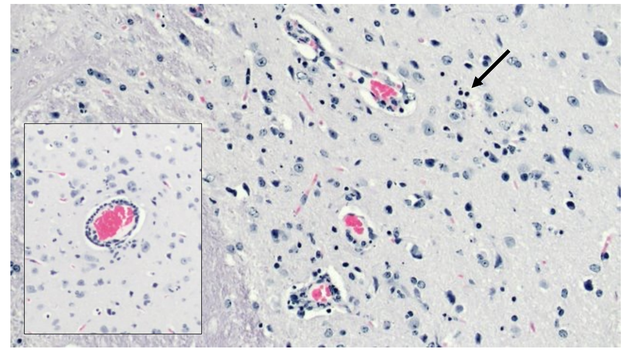


Figure 3. Representative images of mouse brain histopathology, stained with H&E and taken at 20x magnification. Both images are from a mouse euthanized 6-days post-inoculation. Typical brain lesions during acute disease were characterized by perivascular lymphoid cuffing (inset), meningeal lymphoid infiltration, and scattered apoptotic debris (arrow) most notably in the brainstem.

Severity of pneumonia is similar between clinical and asymptomatic mice

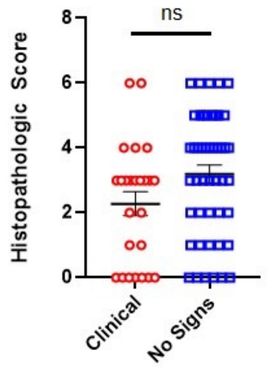


Figure 4. Illustration of histopathologic scores of lungs from mice 6-days post-inoculation. Bars represent mean and SEM. Independent t-test analysis revealed that the difference in lesion severity between mice with and without clinical signs was not statistically significant.

Meningoencephalitis is most evident in mice with clinical signs

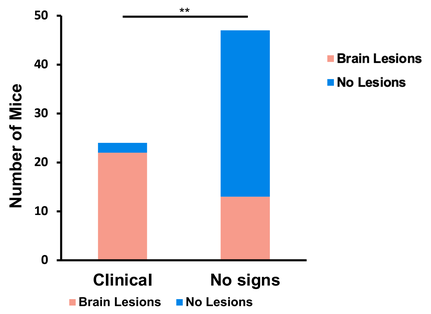


Figure 5. Stacked bar chart showing the distribution of brain lesions between mice with and without clinical signs. Note that nearly all the mice with clinical signs had brain lesions. Also of note, mice with no clinical signs and evidence of meningoencephalitis had very mild to equivocal lesions (data not shown). Chi-square analysis revealed a statistically significant relationship between the presence of clinical signs and lesions (** indicates $p < 0.001$; $\chi 2 = 23.54$)

Proinflammatory cytokine differences primarily in the brain

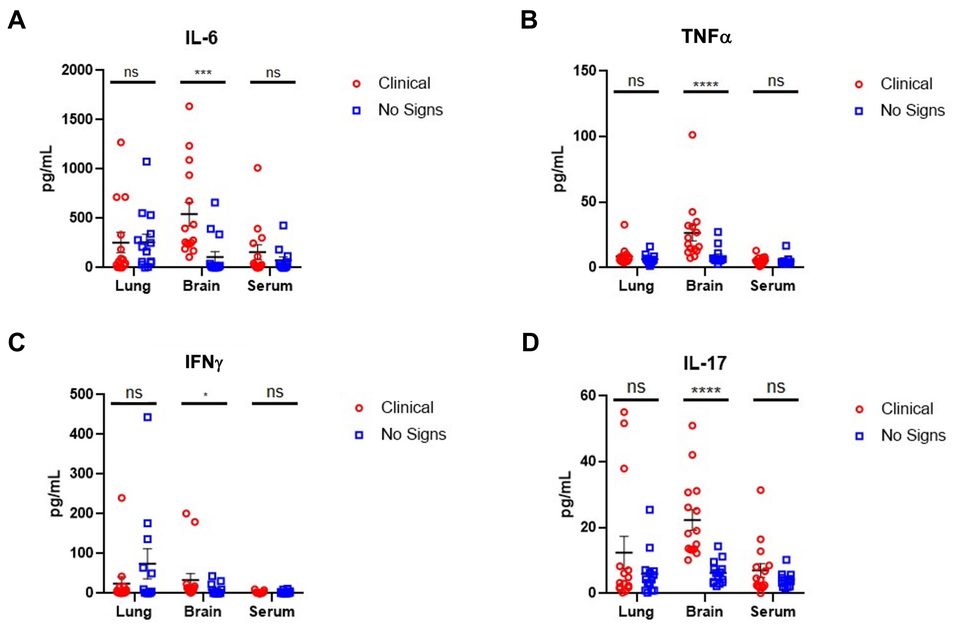


Figure 6. Cytokine levels measured in pg/mL for IL-6, TNFα, IFNγ, and IL-17 in mouse lungs, brain, and serum collected 6-days post-inoculation. Individual Mann-Whitney analyses were done to test significance between mice with and without clinical signs for each sample site. Bars represent mean and SEM. For each cytokine, Mann-Whitney analysis only showed significance in the brain with mice exhibiting clinical signs having significantly higher brain cytokine levels as compared to mice without clinical signs. A) IL-6 (*** indicates $p < 0.0005$). B) TNFα (**** indicates $p < 0.0001$). C) IFNγ (* indicates $p < 0.05$). D) IL-17 (*** indicates $p < 0.0005$).

Conclusions

- Mice exhibiting clinical signs consistently show evidence of meningoencephalitis as well as higher cytokine levels in the brain
- The presence and severity of pneumonia did not differ markedly between animals with and without clinical signs and showed evidence of resolution in mice that survived.
- Collectively, these findings suggest a correlation between brain lesions and clinical disease.

Future Directions

- Pending apoptosis stain (cleaved caspace-3) to further characterize brain lesions
- Further histological analysis at different time points
- Further analysis of cytokine data

Acknowledgements

This project is funded by the Mutant Mouse Research and Resource Center. The stipend for Nikki Seilhamer is supported by the Kent Tomazi Memorial Research Fund in Veterinary Medicine and an endowment established by IDEXX-BioAnalytics. We would also like to thank Alex DeWitt and Chris Johanning for their assistance with mice health checks and necropsies.