Neonatal Mouse model of SARS-CoV-2 and Variants of Concern to Evaluate Therapeutics.

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Abstract

Since first reported in 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is rapidly acquiring mutations, particularly in the Spike protein, that can modulate pathogenicity, transmission and antibody evasion leading to successive waves of COVID19 infections despite an unprecedented mass vaccination necessitating continuous adaptation of therapeutics. Small animal models can facilitate understanding of host-pathogen interactions, target selection for therapeutic drugs, vaccine development and the safety of therapeutics. We and others have shown that the expression of human ACE2 is required to ensure susceptibility to SARS-CoV-2 infection in mice. This study developed a model of SARS-CoV-2 infection to study pathogenesis and the immune response to infection in neonates. Strikingly, challenge of neonatal hACE2tg mice with SARS-CoV-2 Variants of Concern, which are defined by mutations in the Spike proteins (SARS-CoV-2-α, -β, Y, Δ or Ω), resulted in more rapid and severe disease with severe neurological damage, even with significant lower inoculation titres. Further, this model provides a platform in which the safety and efficacy of SARS-CoV-2 therapeutics, particularly monoclonal antibodies targeting the Spike protein can be evaluated. Prophylactic treatment with monoclonal antibodies targeting the receptor binding domain (RBP) of this protein resulted in protection from infection. These studies at BSL-3 are also used to validate studies using VSV-based pseudoviruses expressing the SARS-CoV-2 spike protein from Variants of Concern, which can be utilized at BSL-2.

Introduction

- COVID-19 is primarily a respiratory disease caused by infection with Coronavirus, SARS-CoV-2
  - 3 – 17 % of patients develop severe pneumonia and acute respiratory distress syndrome (ARDS)
- Cardiovascular, renal and neurological outcomes in approximately 30 % of cases.
  - Anosmia, ageusia, headache, meningitis, seizures
- Fatal in 1 – 8 % of cases. Over 1.1 million dead in the US alone and over 6.6 million dead world-wide.

- Long-term sequelae for patients also observed:
  - "Long COVID" or post-acute sequelae of SARS-CoV-2 infection (PASC) symptoms include: fatigue, dyspnea, changes in smell, cognition ("brain fog"), and sleep patterns.

- Vaccines, therapeutics and tests for SARS-CoV-2 (COVID-19) developed, authorized for emergency use and approved in ground-breaking time, with the first vaccine being approved for emergency use in December, 2020.

- SARS-CoV-2 requires the receptor human ACE2 for entry into host cells via the viral Spike protein.
  - As such only species with ACE2 sufficiently similar to human ACE2 or genetically modified mice that express human ACE (under control of the K18 promoter) are suitable as animal models.
  - Intranasal inoculation with SARS-CoV-2 is required to induce disease.
- Variants of Concern are largely defined by mutations in the viral Spike protein.

- Many neutralizing monoclonal antibody (mAb) therapeutics target and ACE2::Spike interactions, blocking entry of the virus into the cell.
  - As Variants of Concern evolve, new variants have escaped neutralization by most authorized therapeutics
  - As such new antibody-based therapeutics and small molecule anti-viral compounds will need to be developed and tested to against emerging variants to determine efficacy as well as safety.

Results

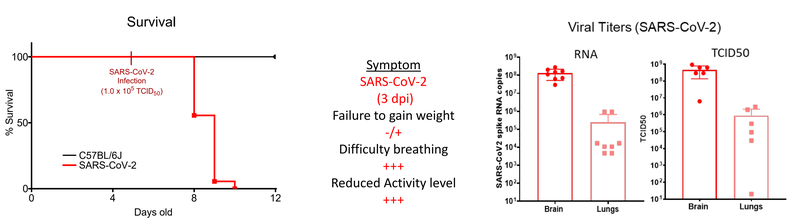
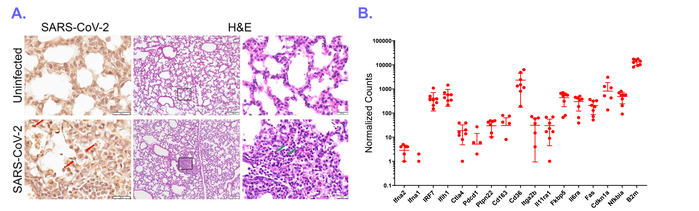


Figure 1. B6, hACE2 Tg mice succumb to SARS-CoV-2 infection. Animals infected with SARS-CoV-2 (WA2020 strain) rapidly develop symptoms within 3 days post-infection (dpi) and succumbed to disease by 5 dpi (age P10). Both viral RNA (as quantified by qPCR for Spike protein expression) and Tissue Culture Infectious Dose 50 ($TCID_{50}$) indicate significant infection in the brains and lungs of susceptible animals at 3 days post-infection (dpi). We were not able to detect the presence of virus in other peripheral organs tested (blood, kidney, spleen, liver).

Results



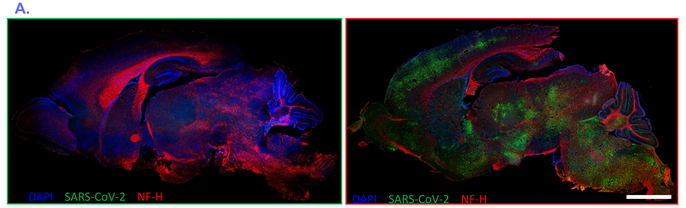


Figure 3. Neuroinvasion of SARS-CoV-2. A. SARS-CoV-2 spreads throughout CNS. **Left**, brain from age-matched uninfected mouse; **Right**, brain from mouse inoculated with Infectious clone of SARS-CoV-2 (WA2020), expressing GFP, to track virus infection (Green) at 3 dpi. IHC staining for neurofilament heavy chain (NF-H, Red). SARS-CoV-2, following intranasal inoculation, spreads quickly throughout the CNS. Scale Bar = 1 mm. **B.** Immunohistochemistry for astrocytes, including Bergmann glia of the cerebellum (GFAP+ cells), combined with SARS-CoV-2 indicates an absence of SARS-CoV-2 infected astrocytes. **C.** Immunohistochemistry for neurons, using NF-H combined with SARS-CoV-2 confirms neuronal infection, including Purkinje cell neurons and neurons in the molecular layer of the cerebellum. Scale Bars = 50 µm.

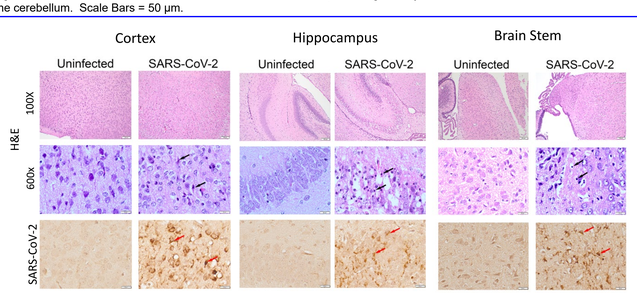


Figure 4. SARS-CoV-2 infection of the CNS results in neurodegeneration. Top rows are representative images of H&E staining from SARS-CoV-2 infected CNS at 100x and 600x magnification at 3 dpi. Degenerative neurons were detected throughout the CNS of infection animals (black arrows), where virus was present, as indicated by immunohistochemistry in the bottom row of panels (red arrows). N=6/group

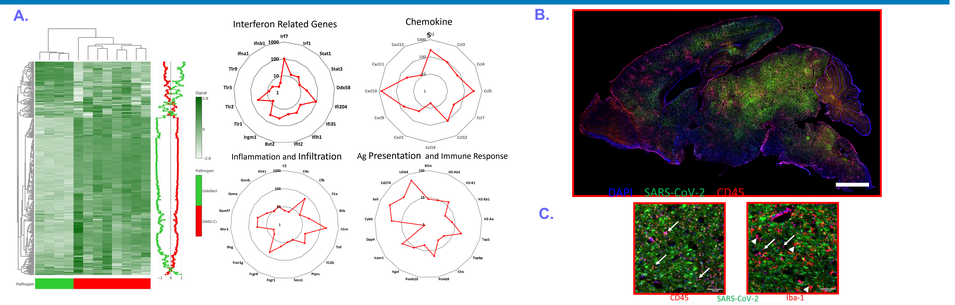


Figure 5. Inflammation, Infiltration and Gliosis. A. Heatmap of gene expression analysis comparing age-matched uninfected and SARS-CoV-2 infected (P8, 3 dpi) brains using Nanostring Mouse Immunology Panel at 3 dpi. Radial plots show selected, significantly upregulated genes for: interferon-related genes, chemokines, inflammation and infiltrating cells and Ag presentation-related genes. **B.** Immunohistochemistry using anti-CD45 antibody confirms the infiltration of immune cells at 3 dpi, throughout the brain and especially in virally infected regions. Scale bar = 1 mm **C.** Infiltrating immune cells (left panel, CD45+, red) are abundant in virus infected regions (green) of the brain. Infiltrating lymphocytes are not infected by SARS-CoV-2. Additionally staining with anti-Iba-1 (right panel, red) revealed significant microgliosis in these regions (white arrowheads) as well as infiltrating macrophages (white arrows). Scale Bar = 50 µm N =6/group.

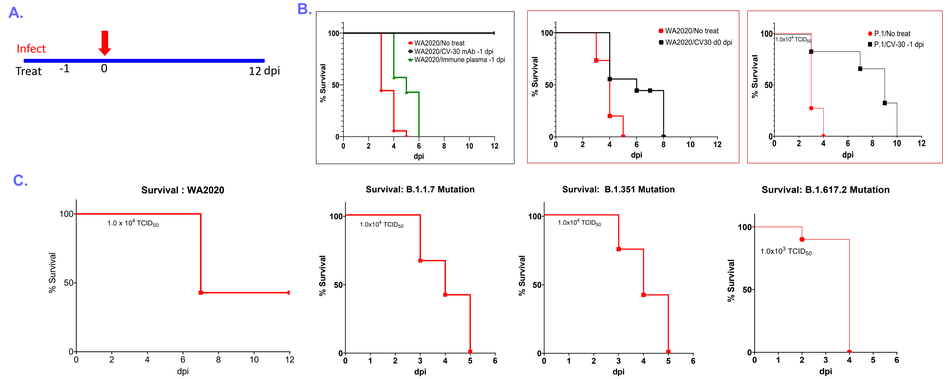


Figure 6. Prophylactic treatment with neutralizing Ab protects from lethal SARS-CoV-2 infection. A. Experimental design of subcutaneous treatment with a neutralizing mAb targeting the Spike protein (clone CV-30) was given to SARS-CoV-2 challenged mice (WA2020 or P.1 variants). **B.** Treatment with CV-30 or human serum from COVID-19 patients was given 1 day prior to inoculation with WA2020 (-1 dpi, left panel) or at the time of inoculation (0 dpi, centre panel). The same treatment failed to protect animals infected with SARS-CoV-2 (P.1, gamma, right panel). **C.** hACE-2 Tg animals susceptible to all variants of concern tested to date. Compared to infectious doses that are less-than-lethal (left panel) when inoculated with SARS-CoV-2 (WA2020), more recent variants of concern: B.1.1.7 (alpha), B.1.351 (beta), P.1 (gamma) and B.617.2 (delta); that have evolved largely due to mutations of the Spike protein, are lethal at 10- to 100-fold lower infectious dose.

Conclusions:

- **Intranasal inoculation of neonatal hACE-2 Tg results in a lethal infection**
  - Untreated Animals succumb to disease within 3-7 days post-infection in a dose and strain dependent manner.
  - SARS-CoV-2 primarily infects the CNS and lungs of hACE-2 Tg mice
    - Virus loads, inflammation and cellular pathology much greater in the CNS than in lung
    - Inflammation, immune cell infiltration and infection result in significant neurodegeneration throughout the CNS.
- **This animal model will serve as an *in vivo* model to test therapeutics targeting SARS-CoV-2, including emerging variants of concern.**
  - Treatment with monoclonal antibodies targeting the Spike protein protected mice from SARS-CoV-2 (WA2020) infection, better than polyclonal serum collected from human COVID-19 patients.
  - However, the same antibody failed to protect against lethal disease when infected with the P.1 (gamma) variant, likely due to mutations in the Spike protein altering the epitope for the monoclonal antibody
  - This loss of efficacy highlights the need for a model in which to test new therapeutics, including: antibody, small anti-viral molecules or combination therapies, to assess safety and efficacy of therapeutics again emerging variants of concern.

- **Using this model in combination with VSV-based SARS-CoV-2 pseudoviruses expressing the Spike proteins of emerging variants, we can rapidly test therapeutics against the Spike protein at BSL-2 (see M. Manangeeswaran poster) and validate these results against the native SARS-CoV-2 variants using this model at BSL-3 where required.**