BSL2-Compliant lethal mouse model of SARS- COV-2 and Variants of Concern to evaluate therapeutics targeting the Spike protein.

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ABSTRACT

Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) variants with increased ability to evade host immune response and increased transmission necessitates continuous testing of novel anti-SARS-CoV-2 therapeutics. The major bottleneck for testing large number of candidate therapeutics is the lack of small animal models that can be used under BSL-2 conditions. To generate the first BSL2-compatible in vivo system we used replication competent, GFP tagged, recombinant Vesicular Stomatitis Virus where the VSV glycoprotein was replaced by the SARS-CoV-2 spike protein (rVSV-SARS2-S). We show that infection of neonatal but not adult, K18-hACE2 transgenic mice (hACE2tg) leads to infection of the lungs and brains. Infection with rVSV-SARS2-S resulted in neuronal infection and encephalitis with increased expression of Interferon-stimulated Irf7, Bst2, Ifi294, as well as CxCL10, CCL5, CLC2, and LILRB4 in the brain and is uniformly lethal. Prophylactic treatment with anti-SARS-CoV-2 spike protein (RBD domain) monoclonal antibody resulted in 100% protection from lethal infection against rVSV-SARS2-S demonstrating the role of SARS-CoV-2 spike protein in infection and disease. Most importantly, our studies show that tropism, disease course and lethality mediated by the SARS-CoV-2 spike protein is comparable to SARS-CoV-2 infection under BSL-3 conditions. To demonstrate adaptability of the model to emerging variants of concern (VOC), we showed that rVSV-SARS2-S viruses expressing spike proteins from VOC (rVSV-SARS2-Spike-α, rVSV-SARS2-Spike-β, rVSV-SARS2-Spike-Y or rVSV-SARS2-Spike-Δ) resulted in rapid lethality (4 vs 10 days) compared to rVSV-SARS2-S. This highlighted the key role of Spike protein in determining the disease course in this model. We propose that rVSV-SARS2-S viruses can be an effective surrogate for the BSL-3 virus to test the potency of therapeutics targeting the spike protein of current or future SARS-CoV-2 VOC under BSL-2 conditions.

BACKGROUND

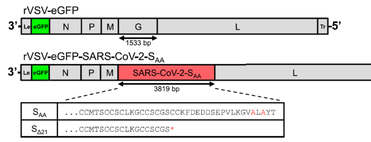
Purpose

- Need for Biosafety Level 3 conditions to conduct SARS-CoV2 research is a major roadblock for screening and rapid development of novel countermeasures.
- The purpose of this study is to develop in-vitro and in-vivo BSL-2 models that can be used to understand SARS-CoV2 entry and pathogenesis and assess the efficacy of therapeutics and vaccines.
- Most of the vaccines and many of the therapeutics target SARS-CoV2 spike protein and this study uses a BSL-2 virus expressing SARS-CoV2 spike protein as the sole glycoprotein on its surface.

Method

We are using replication competent recombinant vesicular Stomatitis Virus where the native glycoprotein (VSV-G) is replaced by the Spike protein of SARS-CoV-2 (Washington isolate). We use K18-hACE2 transgenic mouse.

Construction of VSV-SARS-2 virus in VSV vector



Comparison of SARS-CoV-2 and VSV-SARS-CoV-2-S

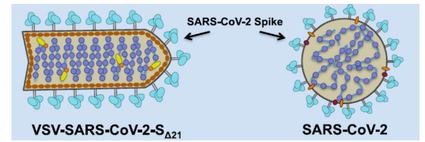
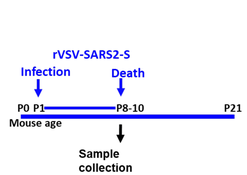
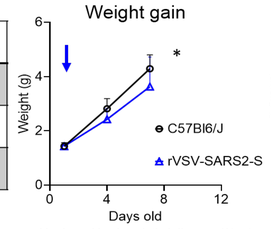
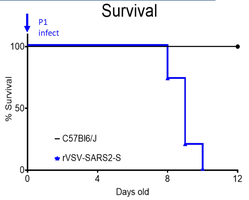


Fig.1. Virus (rVSV-SARS2-S) infection leads to lethal disease in hACE2tg mice



Symptom	rVSV-SARS2-S
Failure to gain weight	++
Difficulty breathing	++
Reduced Activity level	+++





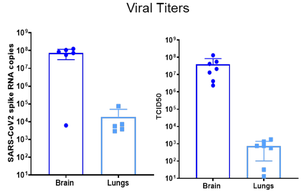
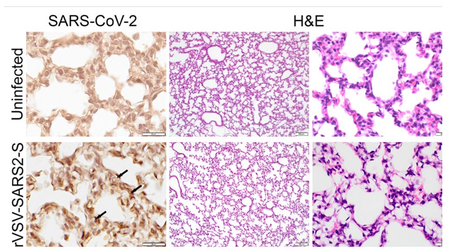


Figure 1. Infection of hACE2tg mice with rVSV-SARS2-S results in infection of lungs and brain and leads to lethal disease. (A) schematic representation of the timeline of rVSV-SARS2-S infection and disease course. Note that mice were challenged on P1 with rVSV-SARS2-S and tissues were collected on P8. (B) Symptoms in hACE2tg mice infected with rVSV-SARS2-S virus. (C-D) Control mice (C57Bl6/J, black, circle n>10) or hACE2tg mice (blue) were challenged intranasally with 10^5 TCID50 of rVSV-SARS2-S (Blue triangles, n>10) and monitored for weight gain (C) or survival(D). (E) Viral RNA titers in lung and brain homogenates of mice infected with rVSV-SARS2-S infected (n=5/group) as assessed by SARS-CoV-2 spike protein specific Taqman assay and infectious virus measured by $TCID_{50}$ assay in Vero E6 cells. Figure shows titers per ug of RNA (qRT-PCR) or half-organ (TCID50). * denotes weight difference between rVSV-SARS2-S and uninfected mice at P8 ($p<0.05$).

Fig. 2. Infection leads to mild lung damage



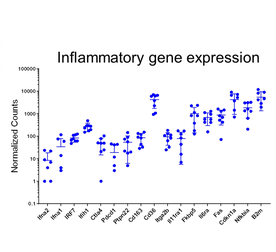


Figure 2. Lung infection in mice challenged with rVSV-SARS2-S virus. (A) Left panels: Immunohistochemistry for Spike RBD. Blue arrows indicate rVSV-SAR2-S infected cells. Right panels show H&E staining of lungs (100 and 600X). Green arrows indicate infiltrating immune cells (image representative of 6 mice/group). (B) Fold-change in selected genes from the lungs of rVSV-SARS2-S (n=8) infected mice at P8 compared to age-matched uninfected control mice as assessed using Nanostring technology (nCounter Mouse Immunology Panel).

Fig. 5. Neutralization and protection

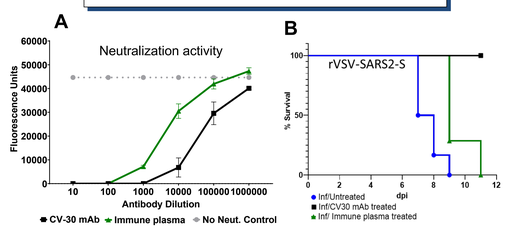
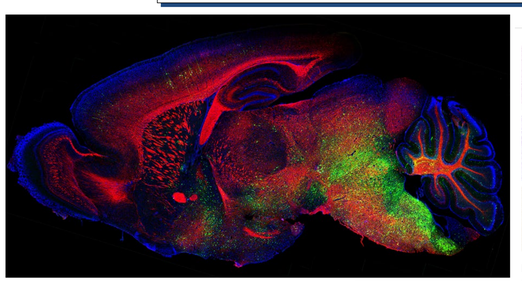
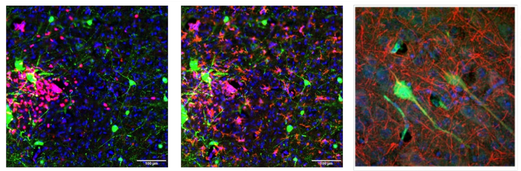


Figure 5. In vitro neutralization and in vivo modeling of antibodies to SARS-CoV-2 Spike bioactivity. (A) Neutralizing activity of an anti-SARS-CoV-2 RBD domain mAb (Black square) and human immune serum pooled from donors recovered from SARS-CoV-2 infection (green triangles) were tested in a micro neutralization assay on VERO E6 cells infected with replicating rVSV-SARS2-S. (B) Human ACE2tg mice were treated intraperitoneally with 2μg/g of CV30 mAb (black line) or 5μl/g of human immune serum (green line) 24 hours before being intranasally infected with 10^5 $TCID_{50}$ of rVSV-SARS2-S (n≥7) and monitored for survival(n≥7/group). Untreated, age matched SARS-CoV-2 (red line) and rVSV-SARS2-S (blue line) Infected hACE2tg mice were used as controls

Fig.3. Neuroinvasion of rVSV-SARS2-S in hACE2tg mice





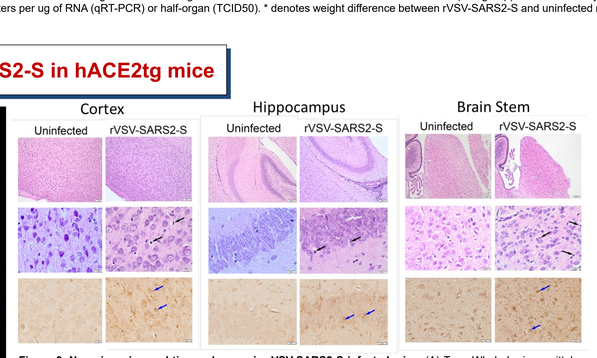
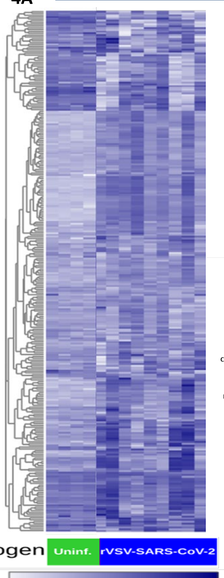


Figure 3. Neuroinvasion and tissue damage in rVSV-SARS2-S infected mice. (A) Top: Whole brain, sagittal section of P8 mice infected with rVSV-SARS2-S virus. Replicating virus labeled with GFP (green), Neurofilament (Red), and nuclei with DAPI (blue). Scale bar:1mm. Bottom: Confocal images of cerebral cortex stained with green (GFP expressed by infecting virus) and Red Indicating: CD45 (infiltrating immune cells), Iba-1 (microglia) or NF (neurons) (N = 6). Scale bar: 25μm.. B. H&E and Spike RBD IHC from infected regions of the CNS. Top: wide-field H&E. Middle: high magnification H&E. Black arrows indicate degenerating neurons. Bottom: IHC staining for Spike RBD. Blue arrows indicate rVSV-SAR2-S infected neurons in these regions (N=6). Scale bar: 100 and 20 μm.

Fig. 4. Gene expression pattern in the brains of infected ACE2tg mice



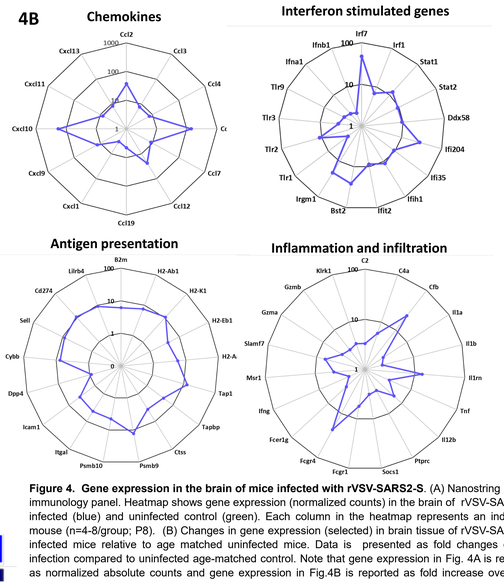


Figure 4. Gene expression in the brain of mice infected with rVSV-SARS2-S. (A) Nanostring mouse immunology panel. Heatmap shows gene expression (normalized counts) in the brain of rVSV-SARS2-S infected (blue) and uninfected control (green). Each column in the heatmap represents an individual mouse (n=4-8/group; P8). (B) Changes in gene expression (selected) in brain tissue of rVSV-SARS2-S infected mice relative to age matched uninfected mice. Data is presented as fold changes due to infection compared to uninfected age-matched control. Note that gene expression in Fig. 4A is reported as normalized absolute counts and gene expression in Fig.4B is reported as fold increase over age-matched uninfected control mice.

Fig.6 Modeling the infection caused by Variants Of Concern (VOCs)

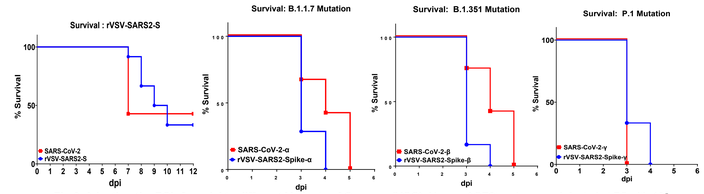
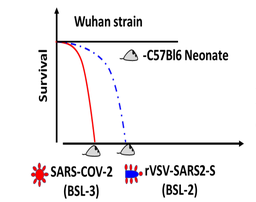
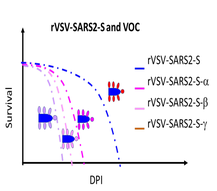


Fig 6. Adapting the BSL-2 model to different Variants of Concern (VOC). Human ACE2tg mice were infected at P5 with 10^5 rVSV-SARS-2-S or with 10^4 TCID50 of VSV pseudotype virus expressing the SARS-COV-2 spike protein from SARS-CoV-2$^{\alpha}$, SARS-CoV-2$^{\beta}$ or SARS-CoV-2$^{\gamma}$ and monitored for survival (n=6-10 mice/group).

SUMMARY

- We have developed a BSL-2 neonatal mouse model where the entry and spread are mediated by the SRS-COV-2 spike protein providing a useful tool to test preventive and therapeutic strategies targeting the spike protein in-vivo under BSL-2 conditions.
- This model can be used to investigate the role of spike protein in mediating pathology and spread and assess the possibility of antibody enhanced disease.
- This model can be used to understand mutations due to therapeutic pressure and predict the development of resistance to therapeutics.





		SARS-COV-2	rVSV-SARS2-S
Containment		BSL-3	BSL-2
Lethality		100%	100%
Viral load	Lungs	+	+
	Brain	+++	+++
	Blood/liver/ heart	+/-	+/-
Clinical outcome	Encephalitis w/ IFN & inflam.	+++	++
Model	Viral clearance	+++	+++
	PK/PD	+++	+++
	Existing VOC	+++	+++
	Potential VOC		+++
	Combinations	+++	+++

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