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The Sars-Cov-2 E Protein Induces Pro-Inflammatory Tlr Signaling, Lung Injury And Alveolar Remodeling In The Neonatal Lung

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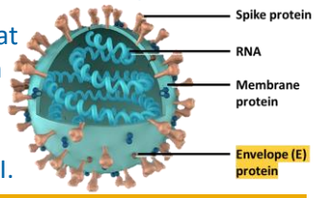
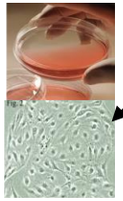
Background and Methods

Background:

- SARS-CoV-2 can cause severe acute lung injury (ALI) in neonates. Neonates with COVID-19 can exhibit a hyper-inflammatory response with vascular injury.
- The mechanisms by which SARS-CoV-2 structural proteins induce ALI in neonates is unclear.
- Emerging data suggest that the envelope (E protein, **E**) of the SARS-CoV-2 activates Toll Like Receptor (TLR)-mediated immune hyperactivation and cytokine storm.
- ✓ We tested the hypothesis that SARS-CoV-2 E protein **(E)** can induce TLR-mediated inflammation and ALI in a neonatal mouse model of ALI.



Primary Human Pulmonary Microvascular Endothelial Cell (HPMEC, ScienCell) experimentation

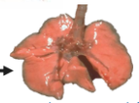


Around 18wks gestation, passages 3 to 4 used.
Control cells or Silencing (si*TLR2*, si*TRL4*) or scRNA (SCBT)
Incubation with 500ng E protein **(E)** (Abclonal), 24hr

Cells processed for:
Trypan blue quantification
PCR, Western Blot (WB)

Mouse (C57BL6) lung experimentation

i.p injection with 10ug E protein **(E)**, typically 48hr



DOL7- RNA, protein → PCR, WB
DOL11- Bronchoalveolar Lavage (BAL)
DOL15- Lung inflations → Morphometry

Lungs harvested for

Results

E protein (E)-induced HPMEC injury, inflammation, and TLR signaling is TLR2-dependent.

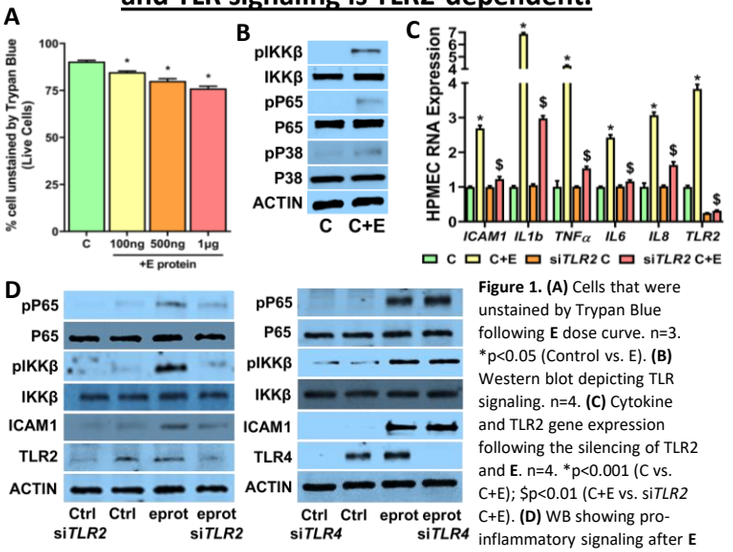


Figure 1. (A) Cells that were unstained by Trypan Blue following **E** dose curve. n=3. *p<0.05 (Control vs. E). **(B)** Western blot depicting TLR signaling. n=4. **(C)** Cytokine and TLR2 gene expression following the silencing of TLR2 and **E**. n=4. *p<0.001 (C vs. C+E); $p<0.01 (C+E vs. si*TLR2* C+E). **(D)** WB showing pro-inflammatory signaling after **E** and siTLR2 or siTLR4.

E protein (E) induces lung cytokine expression and TLR signaling in DOL7 mice.

i.p **E** injection: DOL5

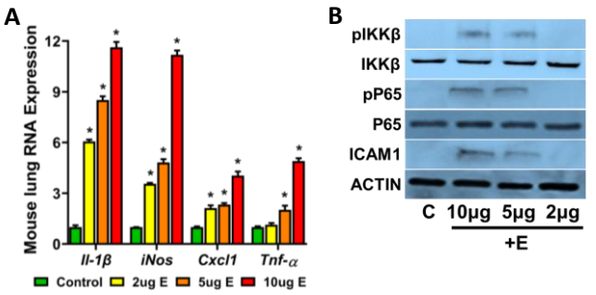


Figure 2. (A) Cytokine gene expression in DOL7 lung lysates by PCR 48hr after **E** dose curve. (n=4). *p<0.01 (C vs. E). **(B)** Western blot after **E** dose curve depicting TLR signaling and ICAM expression. n=4.

Results and Conclusion

Immune cell infiltration and vascular leak measured in DOL11 mice 48hr post i.p E protein (E) treatment.

i.p **E** injection: DOL9

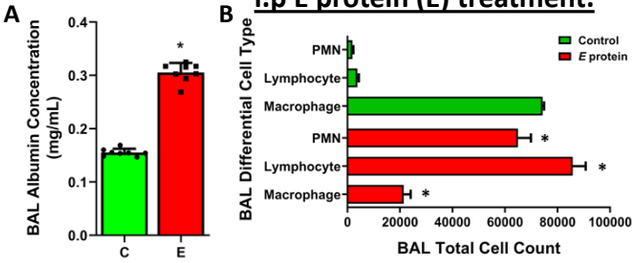


Figure 3. (A and B) Bronchoalveolar lung lavages (BAL) done in DOL11 mice following 48hr **E** treatments, with the levels of albumin quantified **(A)** and the total cell counts with cellular differential shown **(B)**. n=8. *p<0.01 (C vs. E).

The effects of systemic E protein (E) on alveolar simplification in DOL14 mouse lungs.

i.p **E** injection: DOL5

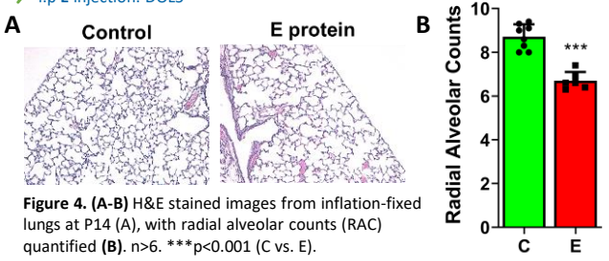


Figure 4. (A-B) H&E stained images from inflation-fixed lungs at P14 (A), with radial alveolar counts (RAC) quantified **(B)**. n>6. ***p<0.001 (C vs. E).

Conclusion:

- The SARS-CoV-2 E protein (**E**) induces TLR signaling, ALI, and alveolar remodeling in the neonatal lung.
- Ongoing *in vitro* studies suggest that **E** induced EC injury and inflammation is TLR2-dependent in HPMEC.
- ✓ *This study provides mechanistic insight into neonatal immune activation and lung injury seen in infants with COVID-19.*

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