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# SARS-CoV-2 Envelope Protein Tolerizes Macrophage Response to Secondary Inflammatory Stimuli

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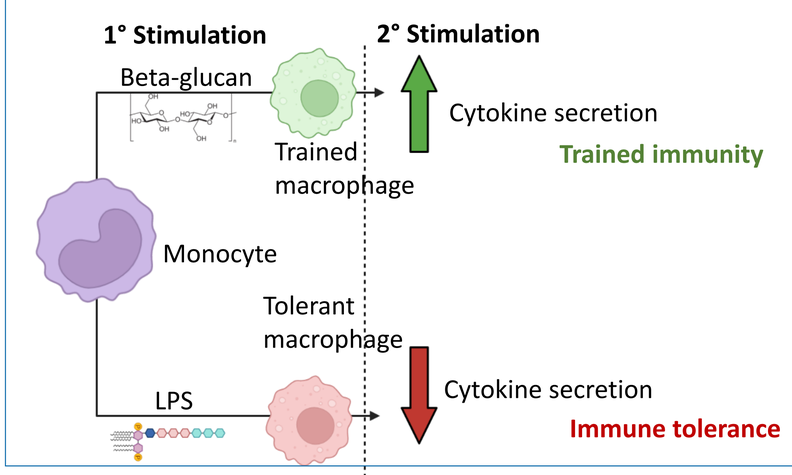
## Abstract

Exposure to bacteria or viruses can change innate immune cells in a way that impacts future immune responses. This exposure results in epigenetic rewiring that either enhances or weakens immune responses to a secondary challenge. One primary mechanism of innate immune cell function is through toll-like receptors (TLRs) that sense a variety of bacterial and viral molecules and cause immune activation. SARS-CoV-2 Envelope protein (E protein) is critical for viral assembly and has been shown to mediate innate immune activation, through engagement of TLR2. Due to its ability to induce inflammation, E protein could play a role in inflammatory disease pathology seen during severe COVID-19. In this study, we determined the secretome of chemokines, cytokines and growth factors from monocytes in response to E protein stimulation and compared this response with other viral antigens and TLR agonists. Next, to investigate if E protein stimulation would modify response to secondary challenge, we performed a primary stimulation of monocytes with E protein followed by secondary stimulation with lipopolysaccharide (LPS) on monocyte-derived macrophages (MDM) 1 week later. Reduced expression of CCL3, CCL4, CCL5, CXCL10, TNF-α, and IL-12 upon secondary LPS or autologous E protein stimulation were statistically reflecting a more tolerant MDM state that is imprinted after E protein exposure. Finally, we demonstrated that immunizing neonatal mice with SARS-CoV-2 E protein induced proinflammatory cytokine secretion and lung tissue inflammation pathology and demonstrated a long-term impact on immune cell function and secondary response to LPS. Thus, SARS-CoV-2 E protein induces macrophage proinflammatory networks that lead to tissue inflammation, but also induce a more tolerant state after return to a resting state that could impact the ability to respond to secondary infection. Further understanding of the molecular pathways that drive this immune activation and tolerance could identify treatment targets to restore immunity after SARS-CoV-2 infection.

## Introduction

- Severe SARS-CoV-2 infection is characterized by overproduction of proinflammatory cytokines (cytokine storm). The mechanism of this is poorly characterized.[1,]
- SARS-CoV-2 envelope (E) protein binds toll-like receptor 2 (TLR2), and elicits expression of pro-inflammatory cytokines in monocytes and macrophages.[2,3]
- Monocytes exposed to certain pathogen-derived molecules can undergo epigenetic rewiring that alters future inflammatory responses.[4,5]
- This rewiring results in enhanced (increased) or tolerized (decreased) secretion of chemokines/cytokines[4,5]




## Hypothesis

**SARS-CoV-2 E protein alters long term function of innate immune cells**

## Experimental Aims

1. Characterize the secretome of human monocytes stimulated with E protein
2. Investigate if macrophages that were primed with E protein as monocytes display an altered response to a secondary LPS challenge.
3. Determine if E protein vaccination in mice alters lung inflammatory responses to LPS injection

## SARS-CoV-2 E protein induces an inflammatory secretome in monocytes

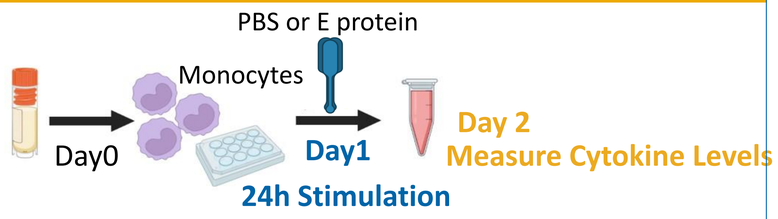
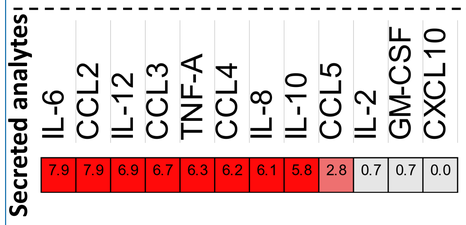
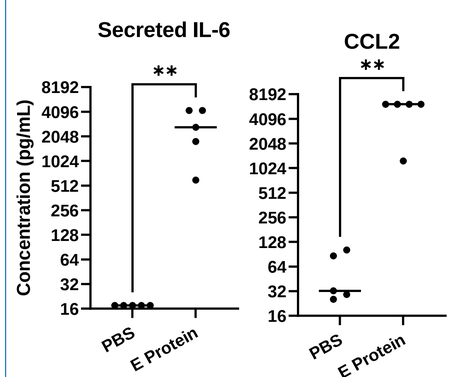



**Fig 1A** We stimulated primary PBMC-derived monocytes with E protein for 24 hours *ex vivo* and measured levels of 25 different analytes secreted by monocytes in the supernatant.




**E protein vs PBS control:**
**LOG 2 Fold-change in concentration**

**Fig 1B** Concentration of 9 analytes were >1LOG2FC in E protein stimulated monocytes vs control. IL-6 and CCL2 were most highly induced by E protein.




**The monocyte secretome after E protein stimulation was composed of cytokines and chemokines that induce inflammation**

## E protein tolerizes macrophage response to secondary LPS stimulation

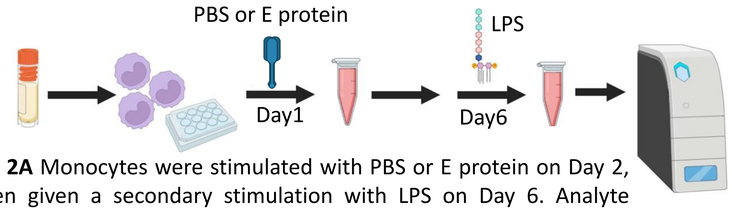
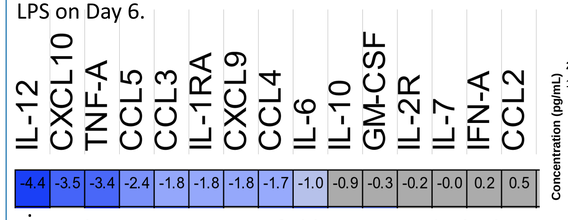
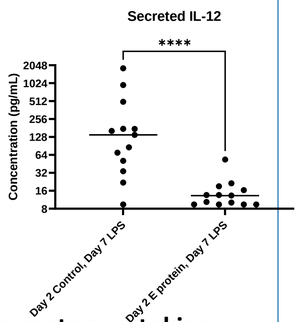



**Fig 2A** Monocytes were stimulated with PBS or E protein on Day 2, then given a secondary stimulation with LPS on Day 6. Analyte levels measured 24h later on Day 7.

**Fig 2B** LOG2 fold-change in concentration of monocytes stimulated with E protein on Day 2 then LPS on Day 6, vs. monocytes given only PBS on Day 2 and LPS on Day 6.




15 analytes were in quantifiable range, and 9 had <-1 LOG2FC in the samples primed with E protein vs PBS. IL-12 showed the greatest decrease.




**E protein tolerized the secretion of proinflammatory cytokines and chemokines in macrophages challenged with LPS**

## Mice vaccinated with E protein display reduced inflammatory gene expression in lung after LPS injection

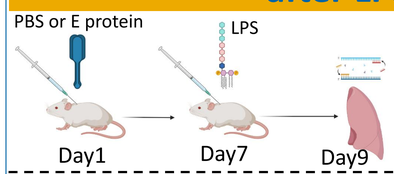



**Fig 3A** Neonatal mice were vaccinated with E protein on Day 1, then LPS on Day 7. On Day 9, lungs were harvested and processed for RNA extraction and qPCR.

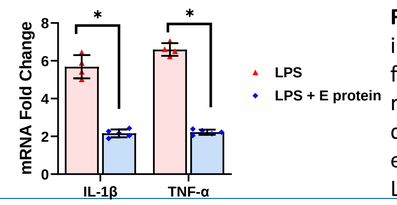



**Fig 3B** Expression of different inflammatory genes in mouse lung following LPS injection. Mice that received both E protein and LPS had decreased proinflammatory cytokine expression vs mice that only received LPS.

## Conclusions/Future Directions

**SARS-CoV-2 E protein induces inflammation and imprints a tolerized response to a secondary inflammatory challenge in human monocytes, and in mouse lung tissue.**

**Future avenues of investigation:**

1. Could E protein be targeted in therapeutic strategies to treat severe COVID-19?
2. Does repeated E protein exposure during severe SARS-CoV-2 tolerize the immune response against a secondary infection?

## Acknowledgements

References: 1. Montazersaheb, S et al Virol J 19, 92 (2022). 2 Zhen et al. Nat Immunol 22, 829–838 (2021). 3 Shahanshah et al eLife 10:e68563 (2021). 4 Moorlag et al Cell Rep. 2020 May 19;31(7):107634. 5 Novakovic Et al Cell. 2016 Nov 17;167(5):1354-1368.e14.

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