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Blocking Respiratory Syncytial Virus infection utilizing decoy cell surface receptor proteins

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Blocking Respiratory Syncytial Virus infection utilizing decoy ICAM-1 receptor proteins

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Abstract

Respiratory syncytial virus (RSV) infections result in a significant number of hospitalizations for young children and the elderly each year, and current infection rates continue to rise, leading to a substantial public health burden. While numerous vaccine candidates are in clinical trials, there is currently no approved RSV vaccine to prevent infection or antiviral treatment after infection. There is an urgent need to find new therapeutics and strategies that reduce severe RSV disease that could improve health and save lives. The RSV fusion (F) protein is critical for binding host cells and mediating infection and is the target of most candidate vaccines. The RSV F protein has been shown to interact with several host-cell surface receptor proteins such as Intercellular Adhesion Molecule 1 (ICAM-1), NCL, EGFR, IGF1R, and infect respiratory epithelial cells. In this study, we confirmed ICAM-1 could bind RSV F protein and inhibit RSV infection via an RSV neutralization assay. Using RSV F protein as bait, we immunoprecipitated RSV F interacting proteins from A549 respiratory cells and performed mass spectrometry analysis to identify novel host proteins that bind RSV F protein. Finally, we engineered a mRNA-lipid nanoparticle (LNP) expressing soluble ICAM-1. The goal is to utilize the ICAM-1 mRNA-LNP as a passive intranasal vaccine that would result in over-expressed soluble ICAM-1 in respiratory mucosal surfaces to function as a decoy receptor that would reduce RSV infection. We are evaluating this therapeutic approach in preclinical models as a prophylactic or treatment after infection. ICAM-1 mRNA-LNP may provide a novel defensive strategy to reduce RSV infection or disease severity and augment future RSV vaccines that become approved.

Introduction

In the US:

- 2.1 million kids see a doctor due to RSV infection.
- 58,000 kids younger than 5 years old are hospitalized with RSV complications.
- RSV infections cause 100-500 deaths in kids under 5 each year.
- An estimated 177,000 adults are hospitalized due to RSV.
- 14,000 deaths are associated with RSV infections annually.

There are limited preventative strategies to inhibit RSV infection
There are limited targeted antivirals for use during RSV infection
(NCIRD, CDC.gov, Li *et al. Lancet* 2022)

Background

The RSV Fusion (F) protein is critical for binding host cells and mediating infection and is the target of most candidate vaccines. RSV-F protein has been shown to interact with several host-cell surface receptor proteins such as ICAM-1, NCL, EGFR, IGF1R, and use these transmembrane proteins to infect respiratory epithelial cells. (Behera *et al. Biochem Biophys Res Comm* 2001, Tayyari *et al. Nature Med* 2011, Currier *et al. PLoS Pathog* 2016, Griffiths *et al. Nature* 2020)

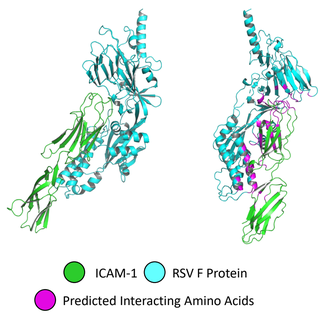
Breast feeding has long been associated with reduced RSV severity, although the mechanism is not understood. Notably, a soluble form of ICAM-1 is an endogenous protein and one of many soluble proteins found in human breast milk. ICAM-1 is an endothelial- and leukocyte-associated transmembrane protein. It is expressed on respiratory epithelial cells, upregulated in response to inflammatory stimulation, and is also the binding site for rhinovirus, the causative agent of most common colds.
(Xyni *et al. Mediators Inflamm* 2000, Attar *et al. Exp Lung Res* 1999, Papi *et al. J Biol Chem* 1999, Greve *et al. Cell* 1989)

Purpose

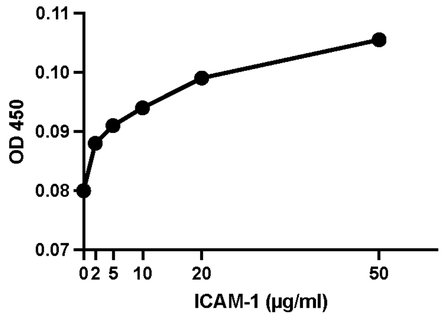
1. Identify RSV Binding Proteins with capability to block RSV infection
2. Identify RSV Binding Proteins with capability to decrease inflammatory cell recruitment
3. Produce highly secreted variant of RSV-blocking protein to prevent infection, decrease viral replication, reduce recruitment of neutrophils

ICAM-1 binds to RSV F Protein

Computation Modeling of ICAM-1 and RSV-F Interactions

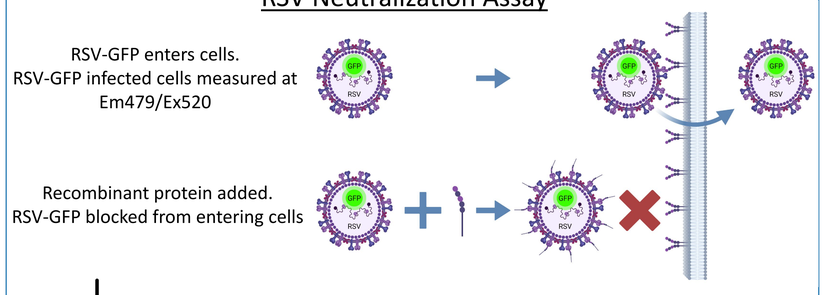


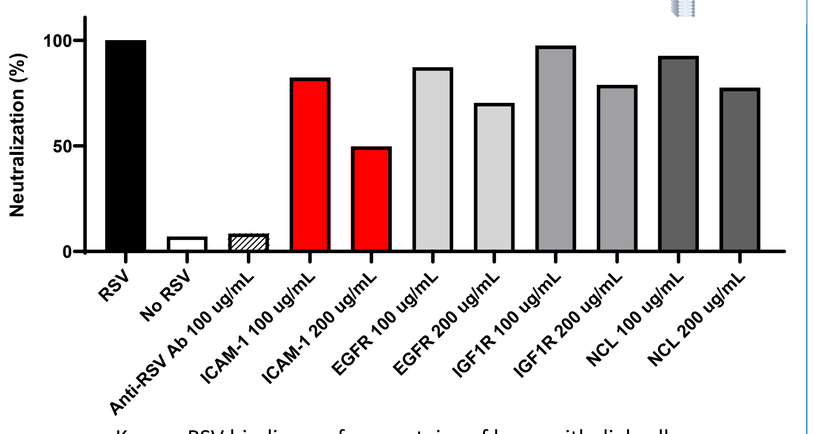
ICAM-1 binding to RSV F protein



Soluble recombinant ICAM-1 blocks RSV infection *in vitro*

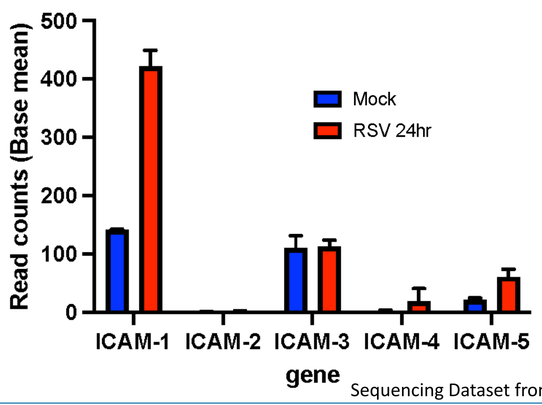
RSV Neutralization Assay





Known RSV binding surface proteins of lung epithelial cells incubated with RSV-GFP prior to lung cell exposure.

ICAM-1 gene upregulated after RSV Infection



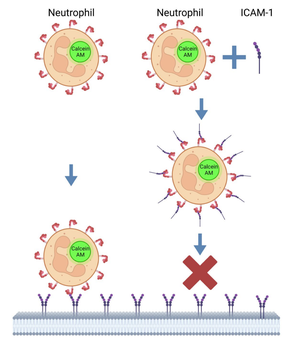
RNA-seq performed 24 hours after RSV infection or mock infection control

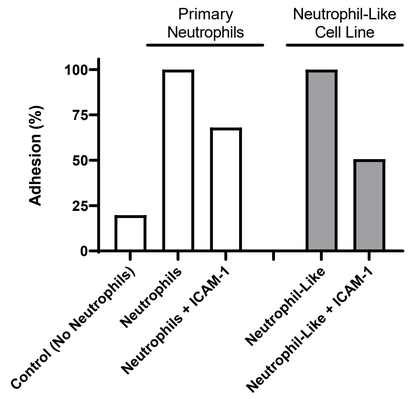
Number of reads of each ICAM family gene reported

Sequencing Dataset from Blanco-Melo *et al.*, *Cell,* 2020

Soluble ICAM-1 reduces pro-inflammatory cell recruitment

Cell Adhesion Assay

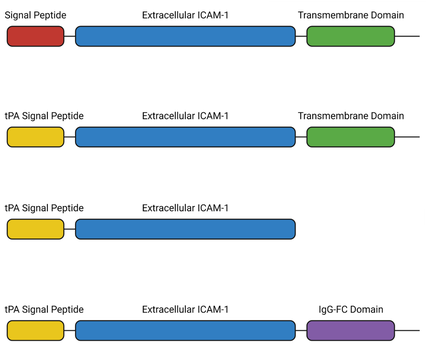


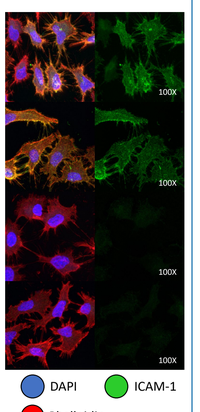


Neutrophil surface protein, CD18, is known to bind ICAM-1 to adhere cells at sites of infection.
sICAM-1 blocks ability of neutrophils to adhere to lung epithelial cells.

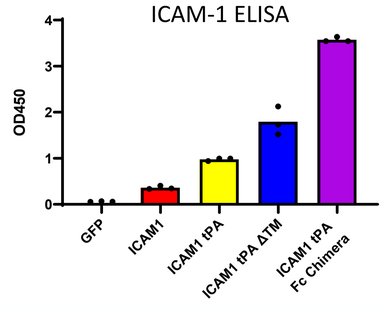
ICAM-1 mRNA Constructs for decoy sICAM-1 secretion

ICAM-1 mRNA constructs:





ICAM-1 ELISA



1. Wild type ICAM-1
2. ICAM-1 signal peptide replaced with tPA signal peptide
3. ICAM-1 tPA construct with transmembrane domain removed
4. ICAM-1 tPA with IgG-Fc Domain replacing transmembrane domain

- mRNA was transfected into lung epithelial cells
- ICAM-1 secreted into supernatant was measured by ELISA
- Surface ICAM-1 was measured with IF Microscopy

Conclusions and Future Directions

1. Identified binding sites between RSV-F and ICAM-1
2. Soluble ICAM-1 blocks RSV infection of lung epithelial cells
3. ICAM-1 gene expression increases after RSV infection
4. Soluble ICAM-1 blocks neutrophil adhesion to lung epithelial cells
5. ICAM-1 mRNA constructs cause ICAM-1 variants to be secreted into supernatant

In the future, we will

- Create Lipid Nanoparticles (LNPs) as carriers of ICAM-1 mRNA Constructs
- Measure the efficacy of ICAM1 mRNA LNPs on preventing RSV Infection or reducing severity and length of infection time *in vivo*
- Determine optimal delivery method of therapeutic for maximum protein production and blocking of RSV

Acknowledgements

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