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Preclinical evaluation of vaccines and antivirals in RSV challenge in BALB/c Mouse models

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Executive Summary

Presently, there is no targeted treatment available for human respiratory syncytial virus (hRSV) infection other than the supportive care to mitigate the signs of and symptoms. Antiviral drug development and RSV vaccine is expected to play a critical role in overall reduction of RSV infections globally. Continued effort in RSV research published in high profile scientific journals has invigorated efforts by larger and smaller pharmaceutical and biotechnology companies to develop antivirals and RSV vaccines. Preclinical development of vaccines is hindered by the lack of clinically relevant rodent models. More importantly, the safety of new vaccines, preclinical pharmacological, safety and toxicology studies should be conducted prior to initiating the clinical studies. Aragen will enable successful IND-enabling preclinical RSV therapeutic development studies by providing appropriate study designs, right animal models, and confirming effective immune response by performing *in vivo* and *in vitro* assays. Our scientists have and will continue to establish the identity, purity, safety, and potency of potential vaccines or antivirals. In this white paper, we present several case studies performed in clinically relevant mouse model to assess the critical characteristics of potential RSV therapeutics. In summary, this article includes in-house as well as client-sponsored study data established over several years using BALB/c mouse models. These studies generated clinically pertinent information for proper screening of new vaccines and antivirals. Aragen will continue to support your RSV therapeutic development effort by providing reliable and effective preclinical services in this important infectious disease area.

Introduction

As per Centers for Disease Control and Prevention (CDC), human respiratory syncytial virus (hRSV) is a common respiratory virus that usually causes mild, cold-like symptoms (1). RSV and its chimeric strains affect infants, adults, elderly worldwide and cause significant morbidity. Aragen continues to serve pharmaceutical, biotechnology and SMEs in their preclinical efficacy and safety studies. Appropriate *in vivo* rodent models enable development of anti-RSV antibodies, small molecules, and vaccines for the treatment of respiratory disease.

RSV is a leading cause of infant hospitalization. RSV infection causes cold-like symptoms and often progresses to bronchiolitis and pneumonia in infants. The cold-like symptoms seen in RSV infected patients appears to be similar to common flu observed in infants. However, infants and older adults exhibit symptoms differently. In infants, the RSV infection might occur at age 2 and remain unnoticed until infant exhibits severe RSV symptoms whereas in adults RSV symptoms are milder and less easy to identify. Unfortunately, there are no targeted antivirals or vaccines readily available to treat or prevent RSV infection. Fortunately, several pharmaceutical and biotechnology companies are striving to develop effective RSV vaccines.

Treatment

Typically, RSV infections go away on their own in a week or two. Virus is contagious, spreads from infected people to others living in close contact with infected persons or areas. There are few steps taken to relieve symptoms, such as managing fever and pain, giving enough fluids, and contacting healthcare provider if the symptoms persist. There is no specific treatment for RSV infection, though efforts are on to develop vaccines and antivirals. Palivizumab, a monoclonal antibody recommended by the American Academy of Pediatrics (AAP) is prescribed to prevent severe RSV illness in certain infants and children.

Present Status of RSV Vaccines

Unfortunately, no vaccine is available against RSV for more than 50 years of effort. Main hurdle for developing RSV vaccine appears to be its multiple mechanisms of evading immunity, hence it reinfects people throughout life with relatively low genetic variation compared to retro (RNA) viruses. The recent availability of structure and antigenic content of the fusion (F) glycoprotein in its metastable untriggered prefusion form (pre-F) and the stable rearranged post fusion form (post-F) gave an impetus to develop vaccine strategies (2). Hence, novel live-attenuated and chimeric virus vaccine candidates and other novel approaches to deliver vaccine antigens have been developed as vaccine products to fight RSV infection (2). Fight against RSV starts with maternal or infant immunization. RSV vaccine should reduce elderly mortality, which is still high. In both populations, vaccine safety is highly critical (3). Palivizumab currently used for treatment of high-risk infants with RSV infection. Developing RSV mice models to measure the RSV-specific neutralizing hu-MoAbs after RSV infection is crucial for early detection and therapeutic intervention (4) in infants.

Preclinical RSV models for vaccine development

The rodent models are preferred choice for most preclinical immunological studies, ranging from simple vaccine testing to the intricate analysis of immunopathogenic responses. Various pathological and immunological parameters of RSV observed clinically have been analyzed using BALB/c mice after intratracheal or intranasal inoculation with RSV-A2 (4). Recent studies have shown that cotton rat is considered a more relevant animal model for preclinical studies on RSV infection than BALB/c mice. Consequently, cotton rats are used to study RSV pathogenesis, anti-RSV drugs, and RSV vaccine efficacy and safety. For example, the cotton rat model was used for pre-clinical evaluation of unglycosylated recombinant E. coli produced G protein (REG) as a potential RSV vaccine (3). A preclinical study showed that bacterially produced REG could provide an economical, safe, and effective broad protective vaccine against RSV disease (3).

Highlights of RSV preclinical Services at Aragen

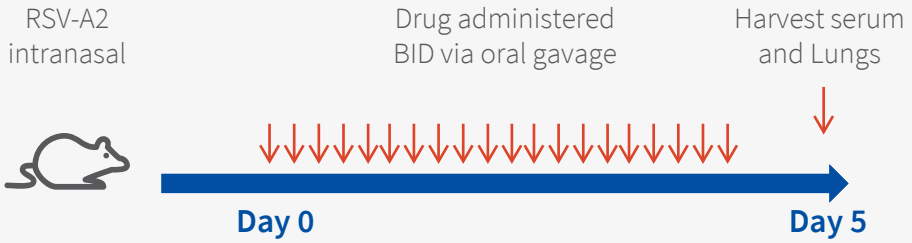
- Experts with several years of expertise, delivered over 100 successful RSV studies (30-150 animals/study)
- A wide range of readouts available with regular updates throughout study

- Flexible and customizable assays performed in RSV models. We have tested small molecules, large molecules, and vaccines for past and existing clients/ partners
- Willing and able to develop RSV models as per client specification with customized studies

Salient features of BALB/c Mouse model

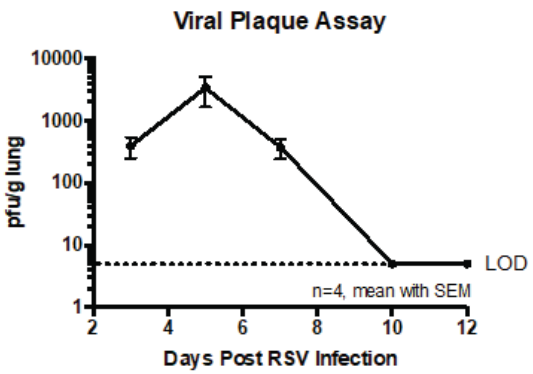
- Semi-permissive for RSV-A strains (not useful for RSV-B)
- Proof of concept studies
- More reagents required for immune readouts
- Non-USDA covered species
- Small amount of test compounds required

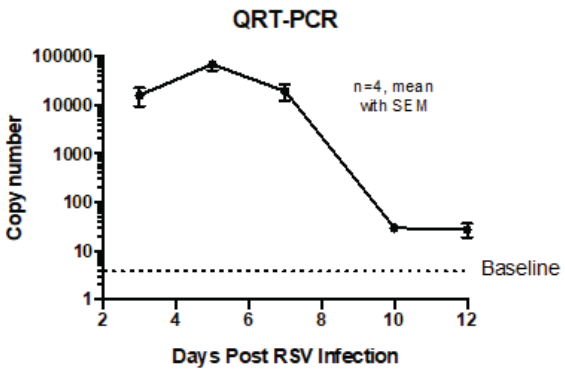
BALB/C SMALL MOLECULE -STUDY DESIGN



- Animals from Aragen approved vendor with dedicated room for study
- Clinical parameters
- Daily body weights
- Daily observations and report for morbidity and ambulatory discomfort
- QC by operators
- Ex-vivo parameters
- Serum processing
- Lung weights
- Viral plaque assay
- Additional readouts: qPCR, ELISA/Bead Assay for Cytokines, BAL processing, leukocyte counts, differential counts
- References

Case study 1: Measurement of RSV replication in BALB/C MICE- Time Course

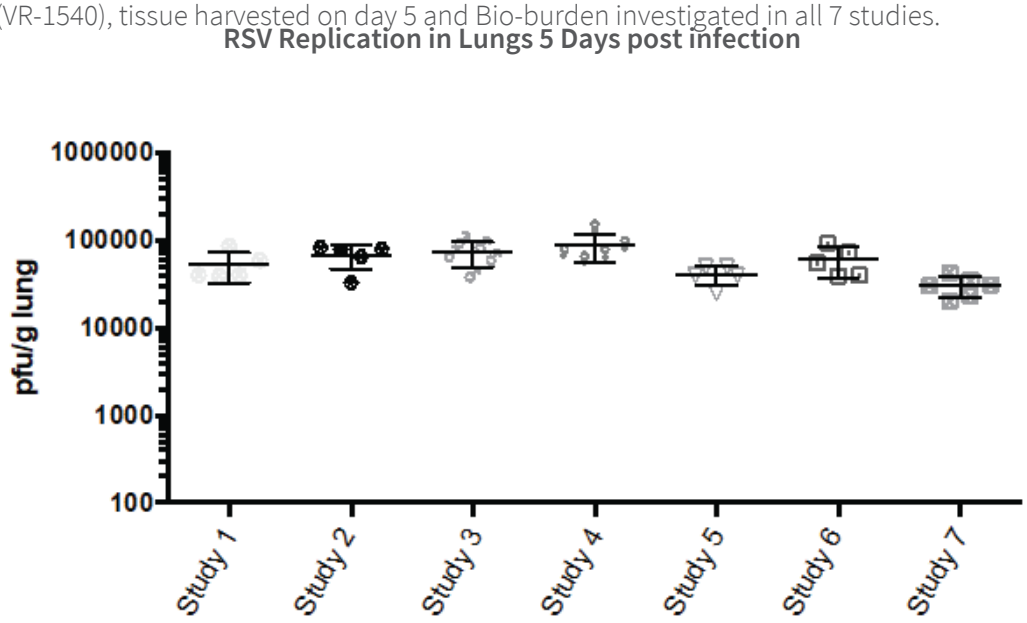




Female BALB/c mice, 6 weeks old were intranasally infected with RSV strain-RSV-A2 Long (ATCC: VR-26). The peak RSV replication was seen on Day 5 post infection (by viral plaque assay). Live virus was not detected in lungs on day 10 post infection. However, viral RNA was detected at day 12 post infection (QRT-PCR method).

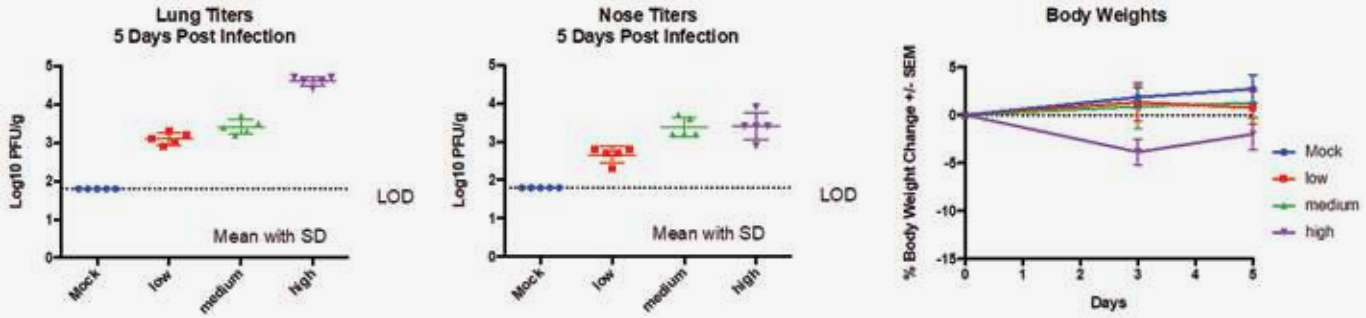
Case study 2: Consistent bioassay performance across studies with RSV-A2

Female BALB/c 6-8 weeks old mice were intranasally inoculated with RSV-A2 (expanded from RSV-A2 ATCC stock (VR-1540), tissue harvested on day 5 and Bio-burden investigated in all 7 studies.



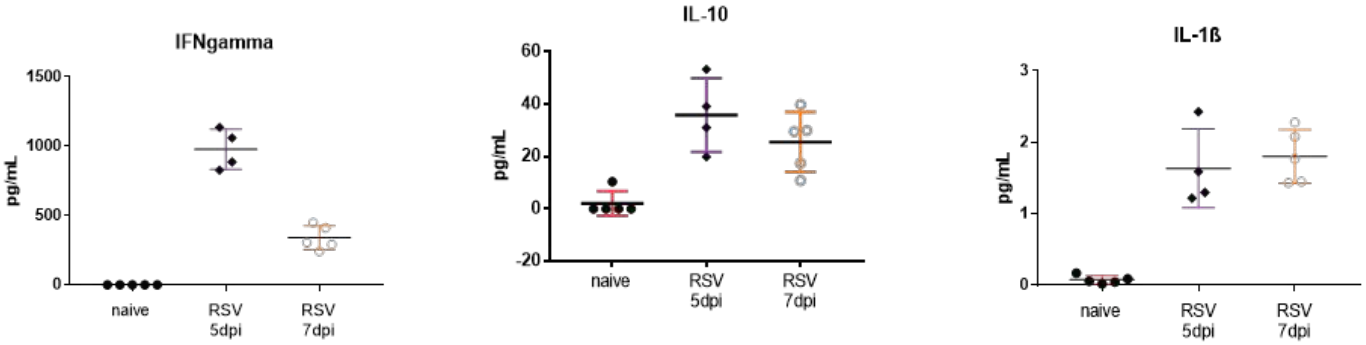
Case study 3: RSV replication in lungs, nasal tissues found after infection with varying RSV doses

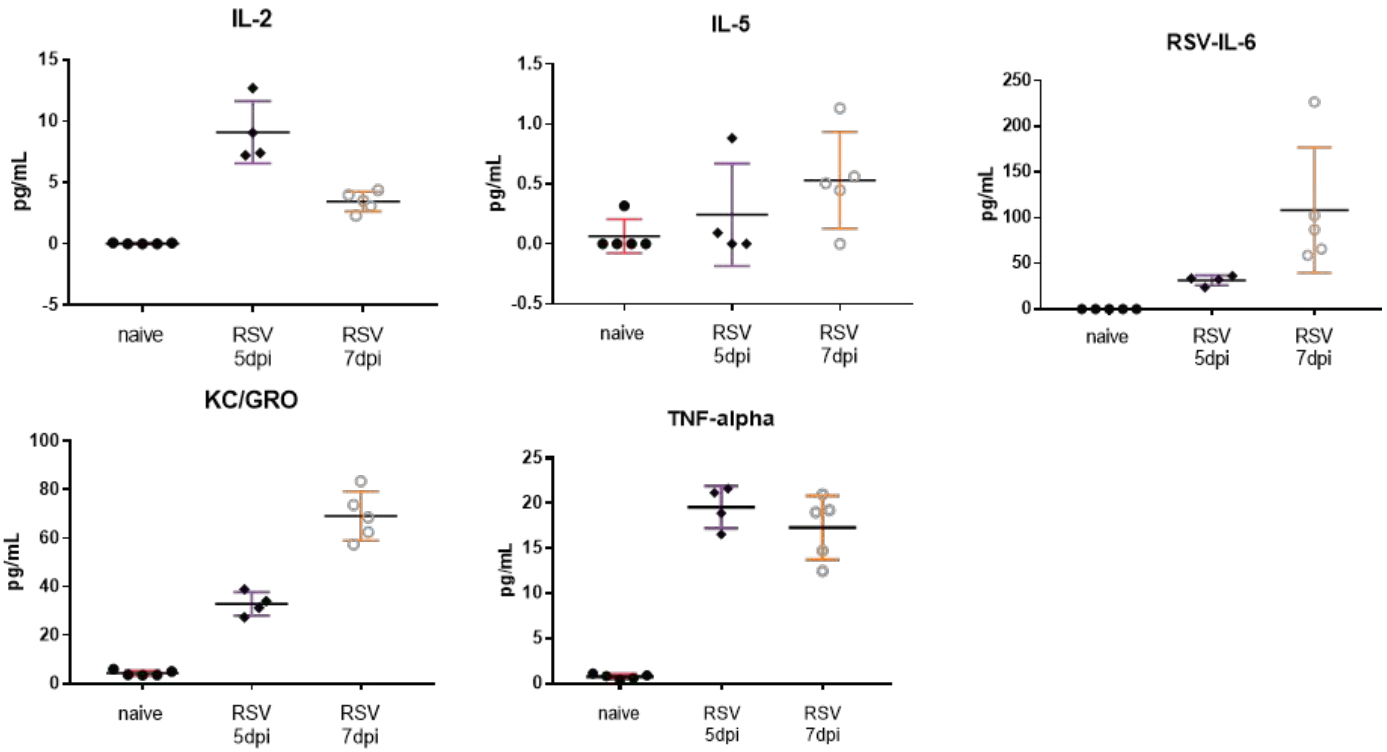
Transient weight loss in female BALB/c, 6-8 weeks old mice intranasally transfected with varying doses of RSV, body weight measured for 5 days. On day 5, lung and nasal tissues were harvested to measure RSV burden. We observed dose dependent significant increase in RSV levels in lung and nasal tissues compared to control group.



Case study 4: Proinflammatory markers at day 5, 7 post RSV-A2 infection were measured in mice.

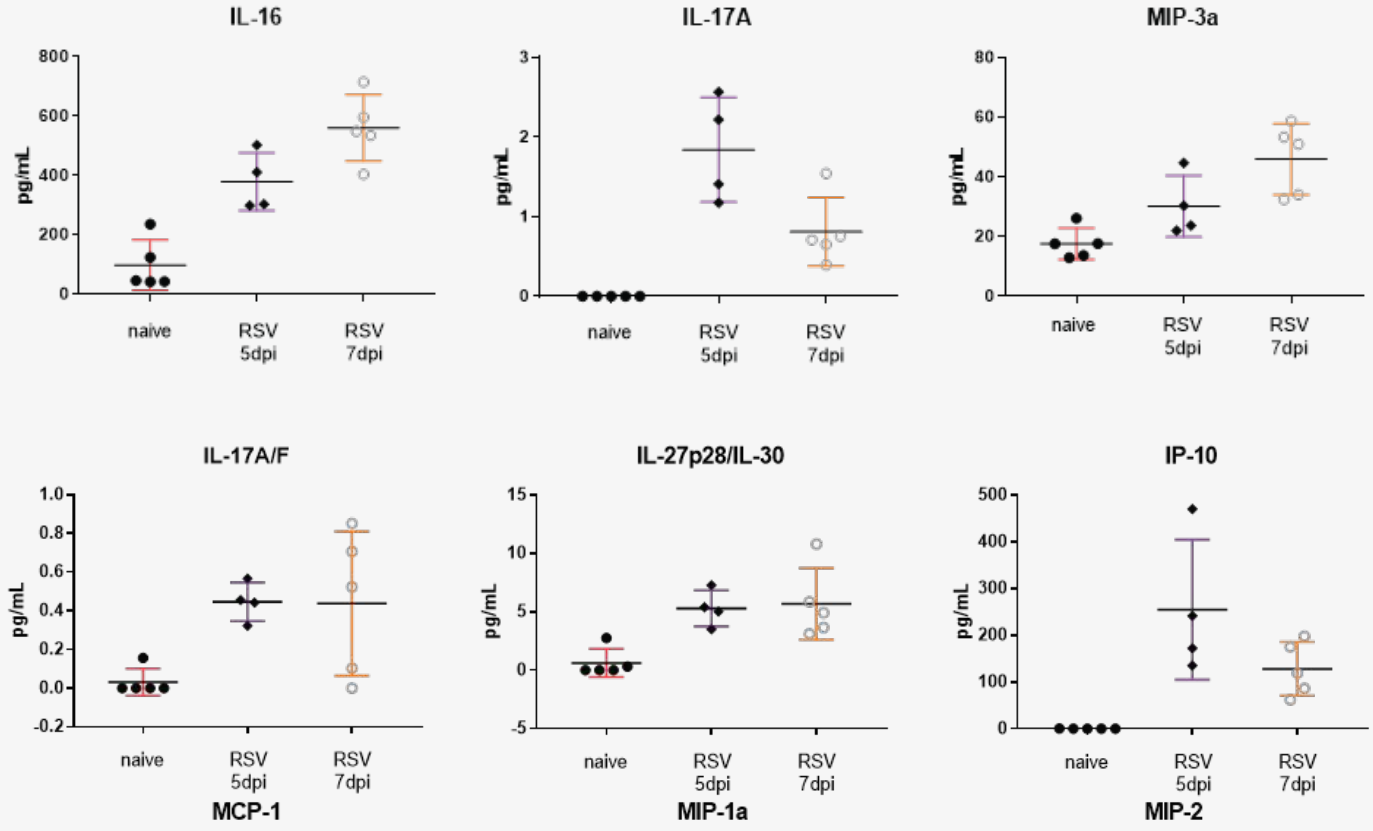
Female BALB/c mice, 6-8 weeks old were infected intranasally with RSV-A2, BAL harvested on day 5 and 7, and MSD analyzed in BAL fluid. Expression of proinflammatory markers after RSV infection are shown in the following figures.

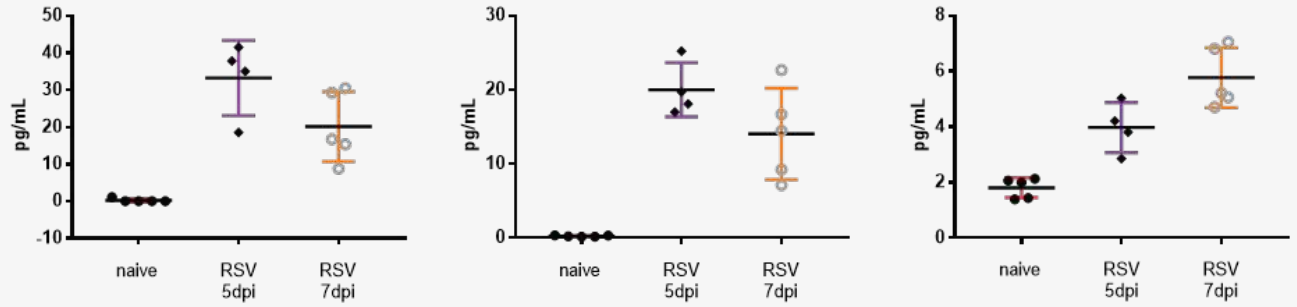




Case study 5: Cytokine and Th17 markers were measured at day 5 and 7 in RSV-A2 infected mice.

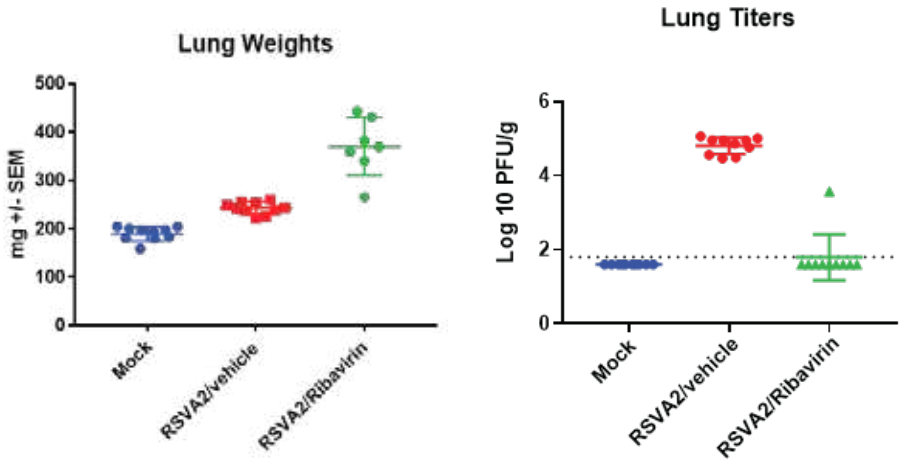
Female BALB/c mice, 6-8 weeks old, were intranasally infected with RSV A2, BAL harvested on day 5 and 7, and MSD analysis performed in BAL fluid. Expression of cytokine and Th17 markers after RSV infection are shown in the following figures.





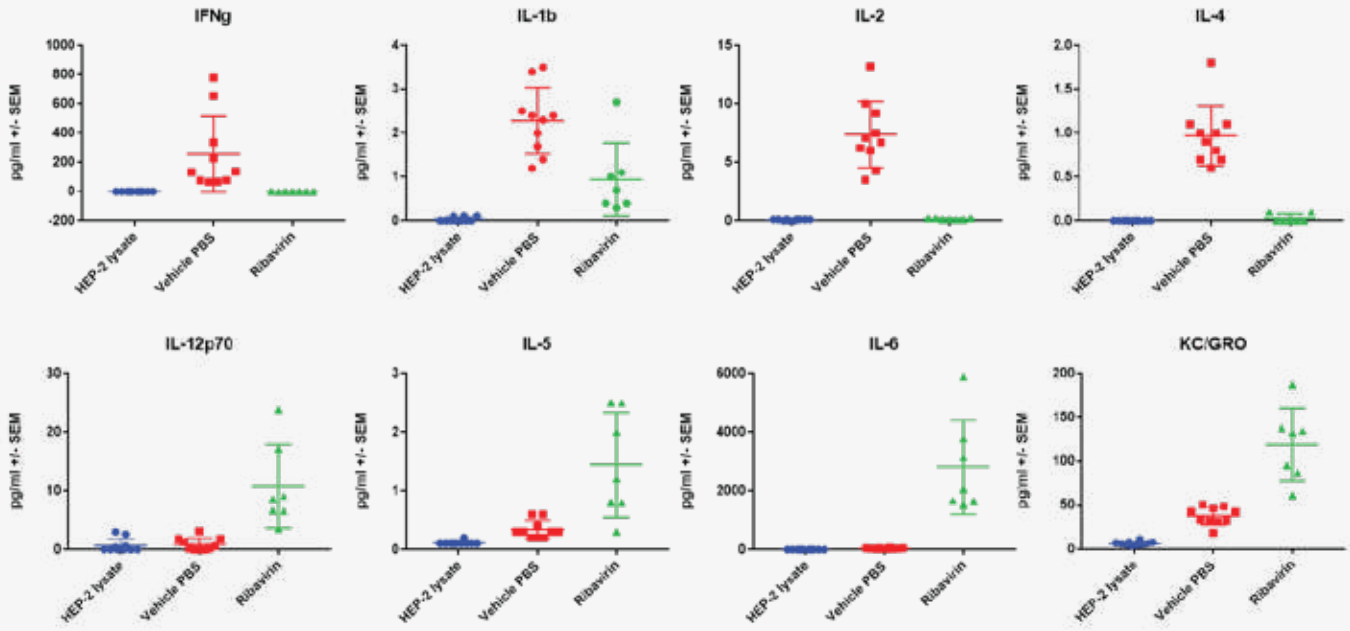
Case study 6: Effect of Ribavirin on RSV-A2 infection in 8-Weeks-old BALB/C female mice.

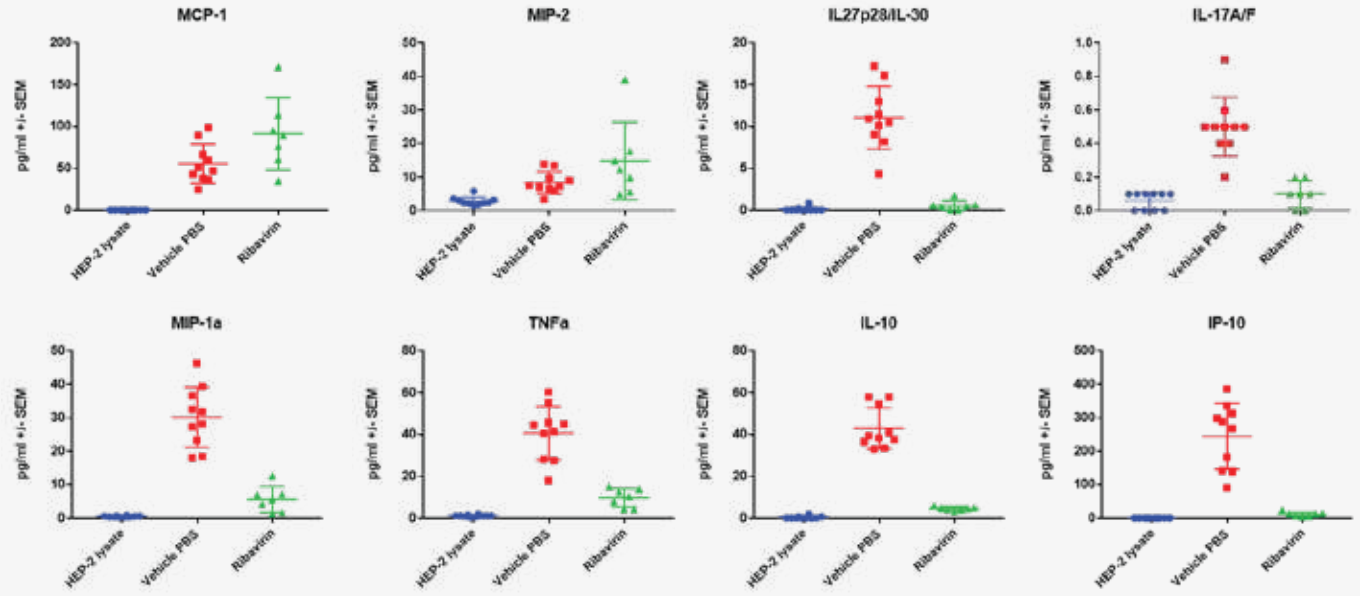
BAL cell counts and BALF MSD analysis using U-plex kit were performed in untreated and treated (Ribavirin) mice. Decrease in lung weights were slightly increased and RSV titer decreased in Ribavirin treated RSV infected mice as compared to placebo (mock) group.



Case study 7: Ribavirin In BALB/c RSV Model- MSD BALF Cytokine/ Chemokine analysis.

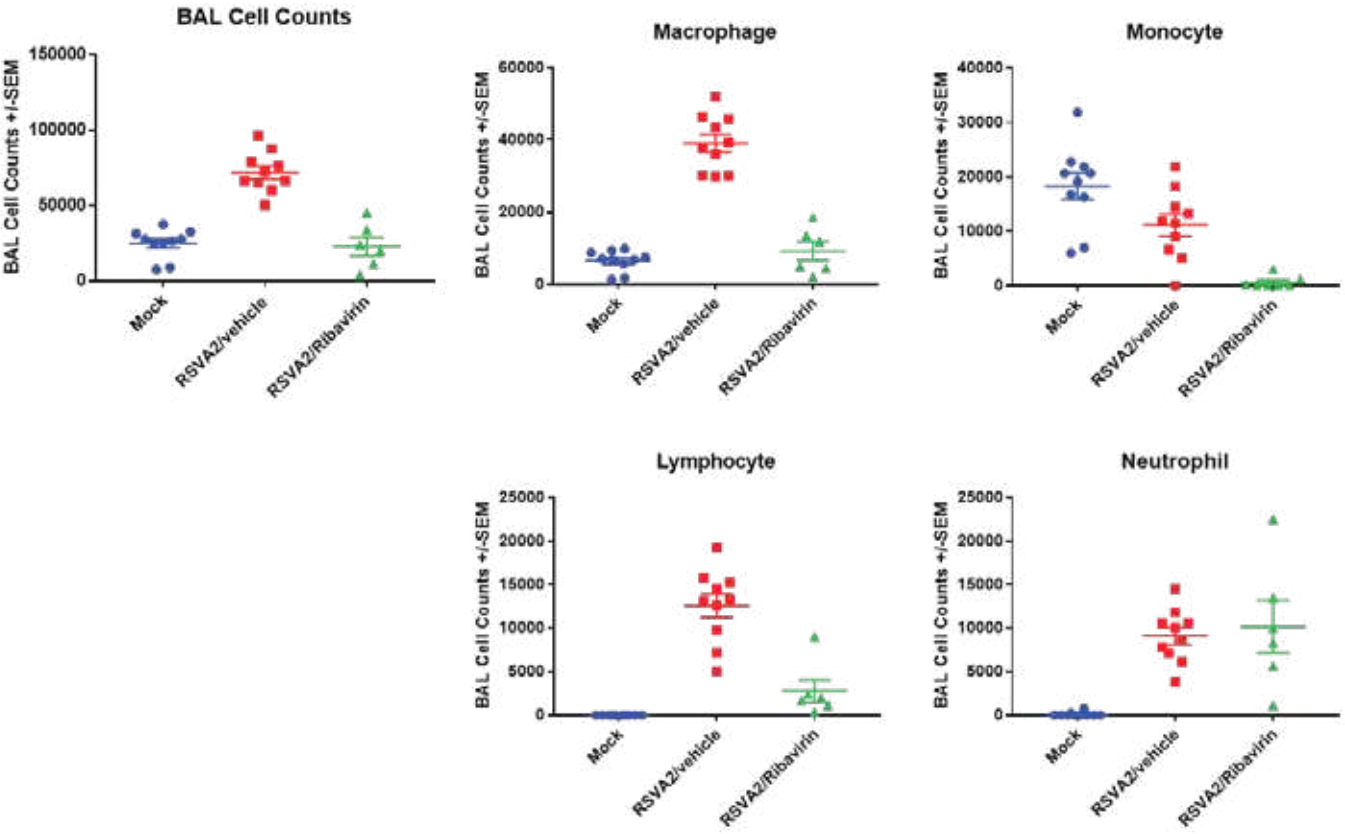
BAL cell counts and BALF MSD analysis using U-plex kit were performed in untreated and treated (Ribavirin) mice. Expression of proinflammatory markers after RSV infection and Ribavirin treatment are shown in the following figures.





Case study 8: BAL Cell counts and differentials in BALB/c RSV Model.

BAL cell counts and BALF MSD analysis using U-plex kit were performed in untreated and treated (Ribavirin) mice. Reduction in counts of BAL cells, macrophages, monocytes, lymphocytes and neutrophils in RSV infected mice samples are observed after Ribavirin treatment, as shown in the following figures.



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Note: References 5-10 pertain to the Aragen case studies.

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About the Author

Dr. Nagendra has held progressive leadership roles in pre-clinical and clinical oncology research and development. He worked at academic centers like-University of Kansas, Cedars-Sinai Medical Center, Mercer University Medical Center, Vanderbilt University Medical center, Anderson Cancer Institute. He led research teams on brain tumor and breast cancer biology. He had extensively published in peer-reviewed journals and secured US, EU and Japan patents. He directed the Human Tissue banking and biorepository and New Animal Facility. Later he worked in pharmaceutical companies namely Dr. Reddy's Labs, Scintilla BioMarc, PPD/ Thermo Fisher Scientific with focus on in vitro diagnostics, clinical pharmacology/ toxicology, and medicinal chemistry aspects of clinical drug development. He is presently a Global Senior Director of Scientific Affairs at Aragen Life sciences, USA.