UNC SCHOOL OF MEDICINE

COVID-19 NC Collaboratory Projects

Final Narrative
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Therapeutics II: Preclinical Studies Of Novel Therapeutic Agents In Mouse Models For Target/Drug Validation, Pharmacokinetic (Pk) Studies, And Efficacy Using Mouse-Adapted Sars-Cov-2 Virus

PI: Ric Boucher, MD
Co-Is: Wanda O'Neal, PhD, Ralph Baric, PhD, Alessandra Livraghi-Butrico, PhD, Samir Kaleda, PhD, Claire Doerschuk, MD, Bev Koller, PhD

This report covers the outcomes and accomplishments for 23-01 C-1 Therapeutics II Mouse Model Testing, which was a collaboration between Drs. Richard Boucher in the Marsico Lung Institute and Ralph Baric in the Department of Epidemiology and Microbiology/Immunology at the University of North Carolina at Chapel Hill.

The overall objective of this grant was to develop a world-class *in vivo* mouse facility that will ultimately allow the testing of therapies to treat COVID-19 disease both at the virus-dominated early damage stage and the late inflammation/repair phase. Additionally, we sought to utilize this facility to explore therapeutic targets, including anti-oxidants, inflammatory mediators, repair mechanisms, and mucus accumulation. We further sought to expand the usefulness of the core by corroborating findings in mice with human COVID-19 autopsy samples. To reach this overall goal, and to prepare us for future research, we have acquired the new equipment required for these studies and formalized the interaction between the Boucher and Baric laboratories with regards to the mice studies (with plans to continue to seek additional funding and published ongoing studies). We are also continuing to make important connections between human and mouse pathologies, focused on both acute disease and the late complications of disease (*i.e.*, the "long-hauler" syndrome).

The following provides a brief summary of progress over the duration of the funding.

Major Research Findings.

The key findings of the research are as follows:

- The mouse adapted SARS CoV-2 virus (maSARS2) developed by the Baric Laboratory was established as a highly relevant model to explore SARS-CoV-2 disease features and treatment. It has the following features that make it particularly useful in this regard.
    - It generates an acute infection in mice that produces respiratory disease.
    - The disease produced in older mice is more severe than in younger mice, recapitulating the human condition. This will allow us to explore specifically the mechanism behind increased susceptibility in the aged.
    - The virus is closely related to the human SARS2 virus, so the underlying biology discovered using the model should be directly applicable to human SARS2.
    - Because the virus infects mice, it can be used in combination with the power of mouse genetics to explore specific genes and pathways important for producing disease. The findings with the different genetic models can then be used to identify new therapeutic targets. Additionally, the model can be used to explore the effect of pre-existing conditions (e.g., chronic bronchitis or chronic mucus obstruction or obesity) on viral pathogenesis and severity, which is of great importance clinically in human COVID-19
    - We have discovered that under some circumstance, the pulmonary disease caused by the virus does not completely resolve, even though the virus in effectively cleared. The features of this progressive pulmonary disease suggest that it might share characteristics with the lungs of human "long haulers," who continue to have respiratory disease and progressive problems (sometimes requiring lung transplant) even after the virus itself has been cleared. Thus:
        - The model will be useful for understanding disease progression (why some people may be susceptible to progression while others are not)
        - The model will be useful to identify key time points in the disease process where different therapeutic options (example steroids, anti-fibrotic therapy, mucolytics) may be most effective.
- Studies correlating human and mouse are highly valuable and have demonstrated:
    - Features of autopsy human lungs are seen in the maSARS treated mice, increasing the translational significance. This is true for both acute and chronic (progressive) changes.
    - Probes for cell-specific genes affected by virus in both human and mouse point to mechanisms of repair that are key to a full understanding of viral pathogenesis.

Datasets and samples produced.

During the short duration of the funded research, we were able to generate several datasets and tissue collections that serve both as a source of present understanding but also as a source of information to guide future efforts. These collections include:

- A whole transcriptome RNAsequencing dataset of the mouse adapted SARS-CoV2 infected mouse lung at two different ages, in both sexes of mice, at sub-lethal and lethal doses, and at two timepoints after infection. This large dataset is being used to explore the genes, proteins, and pathways that are altered after infection. The dataset will continue to help explore genes relevant to viral disease that will serve as therapeutic targets.
- A whole transcriptome RNAsequencing dataset of human airway epithelial cultures at three different age ranges (children, young adult, elderly) at four time points after infection (days 1, 3, 7, 14). The dataset is being used to evaluate age-dependent epithelial processes that regulate and control virus infection and serve as a complement to the in vivo findings.
- A collection of fixed tissue (respiratory samples from the nose to the lung) from maSARS2 infected mice of various types, viral doses, and timepoints after infection to further explore the model and its disease effects. These collections are extremely valuable as they are being evaluated histologically for a wide variety of markers/genes/proteins to gain an understanding of the disease processes. Many histological sections are obtained from a single animal and the sections probed in multiple ways. We are utilizing these sections to explore the cell types that get infected, the fate of the infected cells, the repair process that occurs after the cells are cleared, the inflammatory pathways that control and interact with the infected cells, the disease processes in young versus old mice, the development of progressive disease, and many other questions. The samples are extremely valuable.

A collection of autopsy human lung samples that are used to confirm and extend findings from the mouse lung to ensure that findings are translatable to the human condition. Again, as with the mouse tissue, the human tissue are utilized in a multitude of ways to build an understanding of what the virus is doing to the human lung.

***Equipment purchases*.**

With the funds received, we have purchased several key pieces of equipment that will allow us to be much more efficient in our research moving forward. We have obtained a state-of-the-art HPLC instrument that has been used to monitor biomarkers and drug levels during treatment before and after virus infection; a state-of-the art plate-reader that allows efficient measurement of inflammatory mediators (from BMG); and an aerosol tower (from DSI) that allows mice to be treated via aerosol to the respiratory tract, which we believe is a key tissue to direct therapies.

Publications:

NOTE. A copy of these publications can be found in the accompanying documentation.

One key publication directly resulted from this work. This key publication established the mouse adapted SARS-CoV-2, developed by the Baric Laboratory, as a highly useful virus to study SARS2 pathogenesis and treatment. The valuable contribution of these findings to future research efforts will be discussed below.

Leist SR, Dinnon KH 3rd, Schäfer A, Tse LV, Okuda K, Hou YJ, West A, Edwards CE, Sanders W, Fritch EJ, Gully KL, Scobey T, Brown AJ, Sheahan TP, Moorman NJ, Boucher RC, Gralinski LE, Montgomery SA, Baric RS. A Mouse-Adapted SARS-CoV-2 Induces Acute Lung Injury and Mortality in Standard Laboratory Mice. ***Cell.*** 2020 Nov 12;183(4):1070-1085.e12. doi: 10.1016/j.cell.2020.09.050. Epub 2020 Sep 23. PMID: 33031744.

Several additional publications in high-profile journals occurred during the timeframe of the funding and were supported in part by the provided funds.

Hou YJ, Chiba S, Halfmann P, Ehre C, Kuroda M, Dinnon KH 3rd, Leist SR, Schäfer A, Nakajima N, Takahashi K, Lee RE, Mascenik TM, Graham R, Edwards CE, Tse LV, Okuda K, Markmann AJ, Bartelt L, de Silva A, Margolis DM, Boucher RC, Randell SH, Suzuki T, Gralinski LE, Kawaoka Y, Baric RS. SARS-CoV-2 D614G variant exhibits efficient replication ex vivo and transmission in vivo. ***Science.*** 2020 Dec 18;370(6523):1464-1468. doi: 10.1126/science.abe8499. Epub 2020 Nov 12. PMID: 33184236.

Katsura H, Sontake V, Tata A, Kobayashi Y, Edwards CE, Heaton BE, Konkimalla A, Asakura T, Mikami Y, Fritch EJ, Lee PJ, Heaton NS, Boucher RC, Randell SH, Baric RS, Tata PR. Human Lung Stem Cell-Based Alveolospheres Provide Insights into SARS-CoV-2-Mediated Interferon Responses and Pneumocyte Dysfunction. ***Cell Stem Cell***. 2020 Dec 3;27(6):890-904.e8. doi: 10.1016/j.stem.2020.10.005. Epub 2020 Oct 21. PMID: 33128895.

Dinnon KH 3rd, Leist SR, Schäfer A, Edwards CE, Martinez DR, Montgomery SA, West A, Yount BL Jr, Hou YJ, Adams LE, Gully KL, Brown AJ, Huang E, Bryant MD, Choong IC, Glenn JS, Gralinski LE, Sheahan TP, Baric RS. A mouse-adapted model of SARS-CoV-2 to test COVID-19 countermeasures. ***Nature***. 2020 Oct;586(7830):560-566. doi: 10.1038/s41586-020-2708-8. Epub 2020 Aug 27. PMID: 32854108

Hou YJ, Okuda K, Edwards CE, Martinez DR, Asakura T, Dinnon KH 3rd, Kato T, Lee RE, Yount BL, Mascenik TM, Chen G, Olivier KN, Ghio A, Tse LV, Leist SR, Gralinski LE, Schäfer A, Dang H, Gilmore R, Nakano S, Sun L, Fulcher ML, Livraghi-Butrico A, Nicely NI, Cameron M, Cameron C, Kelvin DJ, de Silva A, Margolis DM, Markmann A, Bartelt L, Zumwalt R, Martinez FJ, Salvatore SP, Borczuk A, Tata PR, Sontake V, Kimple A, Jaspers I, O'Neal WK, Randell SH, Boucher RC, Baric RS.SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract. ***Cell.*** 2020 Jul 23;182(2):429-446.e14. doi: 10.1016/j.cell.2020.05.042. Epub 2020 May 27. PMID: 32526206 Free PMC article.

Additional grants obtained and planned based on provided funding:

To date, two new grants have been awarded based on the funded work. These grants hold a total value of $504,000 and are granted via the Cystic Fibrosis Foundation. Several additional grants are awaiting grant review or are planned for 2021 and are intended for the American Lung Association or the NIH (NIAID). The total pending/planned award dollars = $15,750,000.00. While the funding for these pending/planned grants is not assured, the work will continue and there is a high likelihood of achieving a significant return on investment. The table below lists the features of these grants.

Principal Investigator	Title/Subject Area	Granting Agency	Amount of Award
Funded (award pending)			
Kenichi Okuda	Pathways Balancing SARS-CoV-2 Infectivity/Disease Severity in CF	Cystic Fibrosis Foundation	$125,000/year direct, $280,000 total
Raymond Pickles/Wanda O'Neal	Do mucus secretions and airway inflammation protect the CF Lung from SARS2?	Cystic Fibrosis Foundation	$100,000/year direct, $224,000 total
Pending (submitted, not yet funded)			
Raymond Pickles	Modeling Multi-Virus Respiratory Infections in the Nasal Cavity of Hamsters	NIH/NIAID R21	$150,000 year 1 direct, $427,625 total
Padraig Hawkins (R Boucher, Mentor)	SARS-CoV-2 infection in a mouse model of cystic fibrosis lung disease	Cystic Fibrosis Foundation	$63,350 year 1 direct, $129,050 total
Planned (not yet submitted)			

Richard Boucher/Ralph Baric	Airway epithelial host factors controlling SARS-2	NIH/NIAID R01	$500,000/year direct, $3,887,500 total
Richard Boucher/Ralph Baric	SARS-CoV-2 and development of chronic lung disease: pathogenesis and treatment	NIH/NIAID P01	$1,500,000/year direct, $11,662,500 total
Wanda O'Neal	Pathogenesis and treatment of SARS-CoV-2 lung disease progression using mouse-adapted SARS2 virus in muco-obstructed model	American Lung Association	$100,000/year direct, $200,000 total