Metadescription: Researchers developed a mouse model of COVID-19 using AAV expressing ACE2 receptor, allowing them to study the role of interferon in SARS-CoV-2 infection.

Creating a Mouse Model for SARS-CoV-2 Infection

At the start of the COVID-19 pandemic, researchers had few animal models to study the SARS-COV-2 infection. SARS-CoV-2 infects human cells by interacting with the human angiotensin-converting enzyme 2 (hACE2) receptor before it is internalized. While mice do have an orthologue to hACE2, it does not facilitate infection. SARS-CoV, which also uses hACE2 for infection, does infect mice but only causes mild disease. To overcome this, several labs have created "mouse-adapted" SARS-CoV strains, where the virus has been evolved to better infect mice. However, using these viruses in mice may not fully replicate human SARS-CoV infection.

Towards a mouse model for SARS-CoV-2 infection

To overcome these challenges, a research team led by Akiko Iwasaki at the Yale School of Medicine developed a new model that allowed SARS-CoV-2 to infect mice. They worked with Vector Biolabs's custom AAV production and packaging services to create and produce AAV9-CMV-hACE2, which could then be introduced into the trachea of 6-12 week old mice. Once introduced, these mice could then be infected with SARS-CoV-2 for subsequent research studies.

They chose AAV over other types of viral vectors for several reasons. AAVs are less immunogenic than other viral vectors. This is especially important for studies like this that look at the immune response during viral infection. AAVs can also have a longer duration of expression compared to adenoviruses (ex: months vs. days) so it allows for a broader range of experiments including studying adaptive immunity, longer-term immune responses after vaccination, or responses to small molecule drug candidates.

To test their mouse model, the researchers first looked at whether the model supports viral replication and antibody production after intranasal infection. They found that mice infected with AAV-hACE2 had 200 times more SARS-CoV-2 RNA than control mice. They could also detect infectious viruses as shown using a plaque assay.

Next, the researchers examined lung sections from the mice finding that their pathology represents what is seen in COVID-19 patients. Immunofluorescence staining identified viral nucleocapsid protein in alveolar epithelia. They found an acute inflammatory response as demonstrated by an expansion of inflammatory cells and the activation of lymphoid cells.

In terms of antibody response, the researchers quantified anti-spike IgG using ELISA, finding that AAV-hACE2 mice have a significant antibody response. In a plaque reduction neutralization assay with SARS-CoV-2, they found that a serum dilution of 1:1024 reduced the number of plaques by 75%.

These experiments show that the model using AAV-hACE2 mice supports SARS-CoV-2 infection and that the model is capable of generating an antibody response against the virus.

Role of Interferon in SARS-CoV-2 replication and pathology

The researchers used their mouse model to study the interferon (IFN) and cytokine response to SARS-CoV-2 replication. Type I IFNs are part of the innate immune response and serve as a first line of defense against infections. These interferons trigger the expression of a slew of antiviral genes that inhibit viral replication and activate immune cells. When this response is well-regulated, it helps control the infection and prevents severe disease.

In the current study, they examined RNA sequencing data from infected lungs finding that while the majority of upregulated genes during infection were cytokines and IFN-stimulated genes, type I, II, and III IFNs were not upregulated themselves. This is similar to what's been seen in postmortem lung samples. The top upregulated genes were involved in virus-host interaction, immune response, and immune cell recruitment and activation. When compared to data from patient lung autopsy, they saw that their mouse model accurately accounts for transcriptome changes in COVID-19 patient lungs.

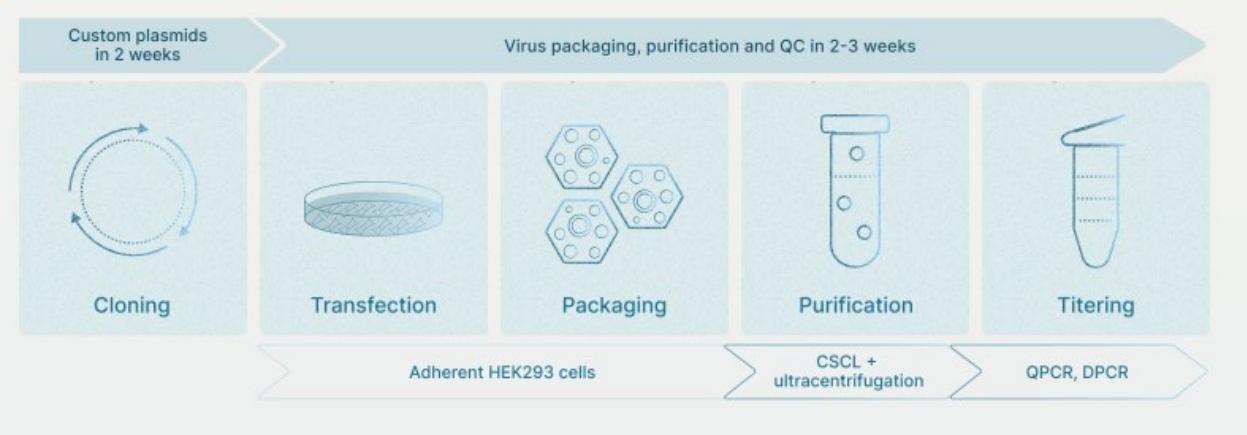
Despite these changes in gene expression, the researchers found that **Type I IFNs don't reduce SARS-CoV-2 replication**. The researchers expressed AAV-hACE2 in wild type mice and mice deficient in the IFN-α receptor or in interferon regulatory factors, and saw that after infection, there was no difference in viral RNA or titer between mice with functional IFN signaling compared to mice without.

In addition, the researchers showed that **Type I IFNs are the drivers behind the pathological response**. In mice deficient of IFN's receptor or its interferon regulatory factors, the mice did not recruit inflammatory cells to the lungs. Wild-type mice expressing AAV-hACE2 had increases in interferon-stimulated genes, cytokines, and chemokines. In contrast, these changes were absent in the IFN and IFN-regulatory transcription factor deficient mice. This is consistent with previous studies that found that Type I IFN signaling drives recruitment and activation of proinflammatory immune cells during viral infection.

AAV Vectors for SARS-CoV-2 Research

Since the publication of this paper in 2020, the work has been cited hundreds of times, and we know more about the role of IFN in the immune response to SARS-CoV-2. Subsequent research has found that the timing of the IFN response in COVID-19 matters. An early IFN response helps viral clearance while a delayed and dysregulated IFN response leads to the cytokine storm and tissue damage that contributes to acute respiratory distress syndrome (ARDS).

At Vector Biolabs, we are proud to have contributed to efforts in developing mouse models for COVID-19 with custom AAVs as well as our off-the-shelf AAVs like AAV controls. End-to-end custom production has a quick 4–5 week turnaround time including designing your AAV, cloning the genes into an AAV backbone, and producing ready-to-inject virus. As an alternative, our AAV packaging service includes packaging customer-provided plasmid into AAV vectors ready for transduction in a 2–3-week turnaround time.



As SARS-CoV-2 circulates today, it undergoes mutations and presents as different variants, some of which are more resistant to IFN activity. A mouse model, such as the one described in this paper, can tremendously help research understand how SARS-CoV-2 infections evolve and how the immune system responds in various contexts like following vaccination or drug exposure. Therapeutically, it's possible that blocking IFN signaling with anti-IFNAR antibodies late in infection can reduce pathology, while administering recombinant IFN early may improve patient outcomes.

To facilitate SARS-CoV-2 research, you can find the following AAV vectors at Vector Biolabs:

- AAVs expressing ACE2
- AAVs for silencing ACE2
- AAVs for expression TMPRSS2
- AAVs for silencing TMPRSS2