Shadowing SARS-CoV-2 Through Mucus and Cilia

Researchers discovered how SARS-CoV-2 invades and spreads through nasal epithelial cells, identifying potential new drug targets to prevent transmission.

BY STEPHANIE DEMARCO, PHD

Whether from a sneezing colleague at the office or a coughing seatmate on an airplane, SARS-CoV-2 wafts through the air and lands exactly where it wants to be: the nose. While the coronavirus can enter the body through any mucus membrane, its most common route of transmission is via inhalation. The virus typically replicates first in the epithelial cells in the nose before spreading throughout the upper respiratory tract and causing COVID-19.

Studies of how SARS-CoV-2 infects cells have revealed that the virus uses its spike protein to bind to the ACE2 receptor on human host cells. The majority of SARS-CoV-2 studies, however, relied on cell culture conditions or animal models that don't always fully model the human upper respiratory tract environment.

To better recapitulate the human respiratory system *in vitro* and reveal how SARS-CoV-2 infects nasal cells, a research team led by Peter Jackson, a cell biologist at Stanford University, and virologist Raul Andino at the University of California, San Francisco developed an organoid system that models the human nasal epithelium. The team identified potential new targets to prevent viral spread using this system (1).

"We wanted to understand how it all worked," said Jackson. "What does it look like for the virus in the beginning of an infection?"

Nasal epithelial cells are decorated with hundreds of little cilia that sense the environment outside of the cell and send signals to the rest of the cell body. Cilia also play a mechanical role in moving the layer of mucus that rests on top of nasal epithelial cells. Mucus traps microbial threats in its sticky mass, and as the cilia beat, they push mucus out of the nasal cavity and into the throat to get rid of it. This acts as the first line of defense in preventing pathogens from infecting nasal epithelial cells, but somehow, SARS-CoV-2 breaches this defense. Jackson, Andino, and their teams wanted to figure out how.

A ciliary beacon

In a paper published just a few months after the start of the pandemic, Jackson and his team reported that ACE2 and its coreceptors localize to the cilia of nasal epithelial cells (2). They hypothesized that SARS-CoV-2 likely entered the nasal cells there.

To test this hypothesis, they infected their nasal epithelium organoids with SARS-CoV-2 and monitored them for three days to see how the virus infected the cells. Using immunofluorescence imaging, the researchers showed that by 48 hours post-infection, SARS-CoV-2 had infected ciliated nasal epithelial cells.

"Virologists tend to just measure RNA and not do microscopy, but the visual part of this was critical in the sense of seeing where the virus was," said Jackson. "We realized that the virus not only was using these ACE2 receptors, but actually that it was binding to the surface of those cilia at a really high density."

Mucin barriers and cilia ladders

To refine their idea of how quickly SARS-CoV-2 binds to cilia after infection, the team relied on the lead author, Chien-Ting Wu's expertise in electron microscopy (EM) to take scanning EM images of the organoids soon after SARS-CoV-2 infection. Wu, who is now a virologist at UT Southwestern, observed virions dotted all over the cilia as soon as six hours after infection.

The researchers wondered why the virus took 24 to 48 hours to infect the cells if they reached the cilia in only six hours. In cell culture experiments, Jackson said, "the virus gets into the cell in about 10 minutes." They hypothesized that the mucus layer, which was absent in cell culture experiments, slowed the virus down.

To crack this mystery, the researchers treated the organoids with mucinase, an enzyme that digests the main component of mucus called mucins, and infected the organoids with SARS-CoV-2. The virus invaded the cells much faster than before.

"When you accept that the mucins are the barrier, what does the cilia do?" Jackson asked.

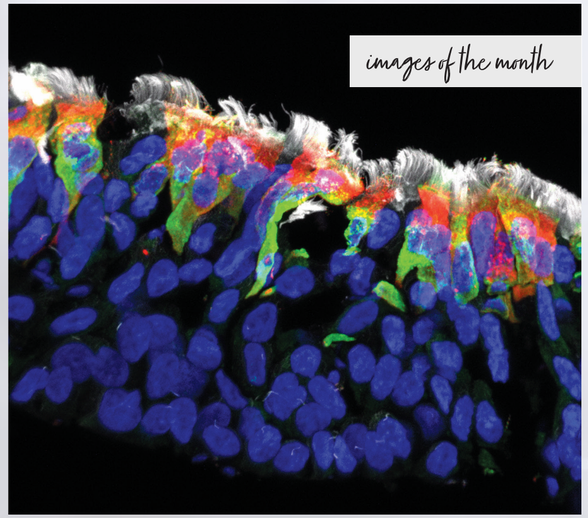
The team then knocked down cilia in the organoids and found that SARS-CoV-2 couldn't enter the cells at all. They even showed that other respiratory viruses including respiratory syncytial virus (RSV) and parainfluenza virus (PIV) also couldn't infect the nasal epithelial cells without cilia.

"That confirmed the model that the virus needed these little portals or ladders to crawl through the mucous," said Jackson. "The cilia are kind of like the ladders that the army in Game of Thrones uses to climb up the walls of the castle and attack."

Microvilli escape routes

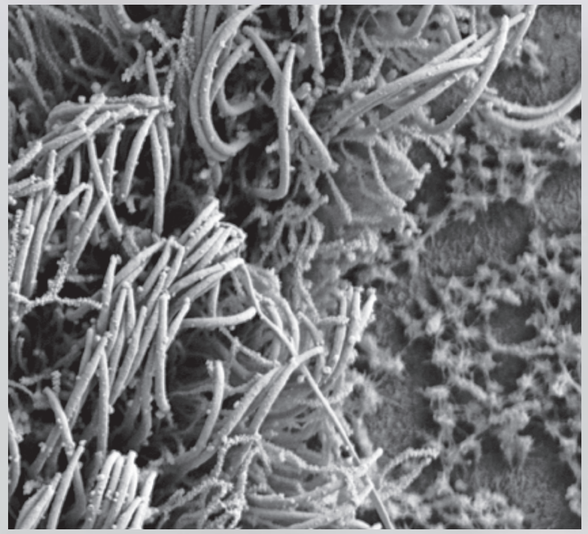
After figuring out how SARS-CoV-2 enters the cells, the researchers wanted to know how the virus climbs back out and spreads to other nasal epithelial cells.

To find out, the researchers used transmission EM to visualize the nasal epithelial cells after infection and noticed that emerging SARS-CoV-2 virions clustered around what looked like brand new structures. Normally, cells can form extensions called microvilli that look similar to cilia but are nonmotile and typically shorter. But the researchers saw that the SARS-CoV-2 infected nasal epithelial cells formed extremely long and more branched microvilli than they had ever seen before. The uninfected cells did not form these structures.



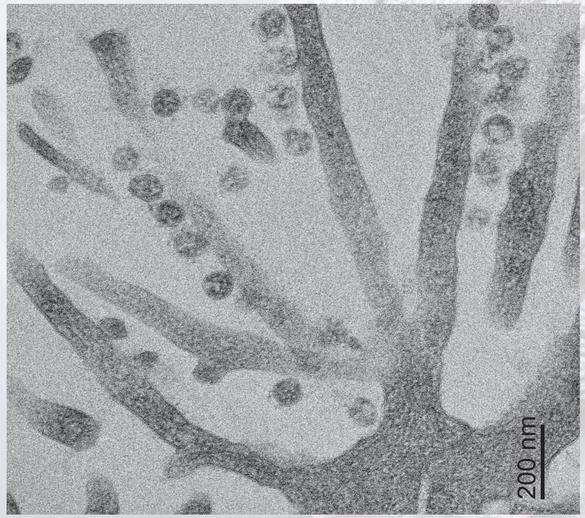
CREDIT: CHIEN-TING WU

SARS-CoV-2 virions, shown by nucleocapsid proteins in red and viral spike proteins in green, invade the cilia, represented by acetylated tubulin in white, of human nasal epithelial cells. Cellular DNA appears blue in this immunofluorescence image.



CREDIT: CHIEN-TING WU

The small dots decorating the cilia in this scanning electron microscope image are SARS-CoV-2 virions. They cling to cilia as early as six hours after infection.



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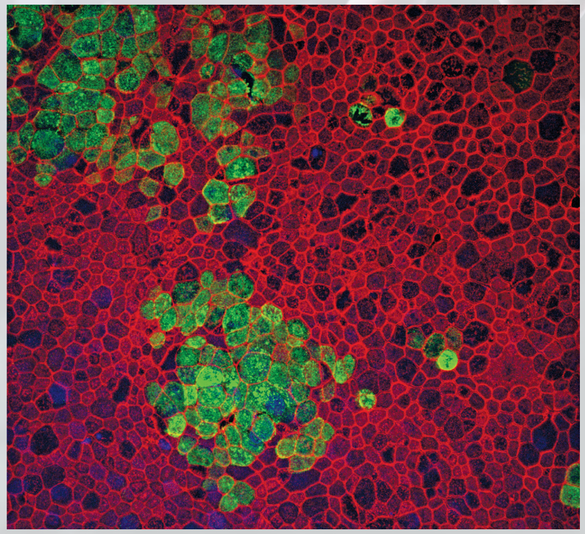
Using transmission EM, the researchers observed SARS-CoV-2 virions — the circular structures — exiting abnormally branched and extended microvilli.



CREDIT: CHIEN-TING WU

In organoids made from PCD patient cells, SARS-CoV-2 — shown by the spike protein in green — can't spread to other nasal epithelial cells, represented by acetylated tubulin in red in this immunofluorescence image.

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>
> *– Peter Jackson, Stanford University*

"If you look at some of the EM pictures, they're really kind of amazing," said Jackson. "When you infect with the virus, the microvilli, they branch, and that's just crazy. I don't think anyone had ever observed that."

Beating cilia spread SARS-CoV-2

SARS-CoV-2 engineered its own way out of cells via the branched microvilli, but the researchers wondered if the virus might still need cilia to spread to other cells. To test this, the team took advantage of nasal epithelial cell samples from people with the genetic disease primary ciliary dyskinesia (PCD). People with PCD can form cells with cilia, but the cilia don't beat synchronously.

"It's like having a bunch of rowers in the boat, but they don't have a coxswain to tell them to beat together," Jackson explained. "Patients who have that disease are very sensitive to infection, so you need to coordinate a beating system in order to be good at clearing viruses and bacteria."

To see if synchronously beating cilia were necessary for SARS-CoV-2 to spread to neighboring nasal epithelial cells, the researchers created organoids using PCD patient cells. Using immunofluorescence imaging, they found that virus-infected cells clustered together in tight groups. Without properly beating cilia, the virus couldn't spread and infect the entire organoid as in organoids with healthy nasal epithelial cells.

Mechanisms and new drug targets

Using phosphoproteomics to profile the infected epithelial cells, the team found that SARS-CoV-2 reprograms the cells to express specific protein kinases called PAK1 and PAK4. When they inhibited these kinases in infected organoids, the microvilli didn't form, pointing to PAK1 and PAK4 inhibitors as potential ways to block SARS-CoV-2 spread to other cells. The main stumbling block in using these inhibitors in humans, however, is their potential toxicity.

"You're targeting a host cell enzyme, not the virus itself," Andino said. But, he added, "Here, the advantage is you might need to use it only for a short time, for few days, so the toxicity may be less of an issue." Blocking viral spread at the start of the infection could work well enough to prevent COVID-19 symptoms and slow transmission of the virus to other people.

Jackson is working to secure additional funding to continue this project and better understand the mechanisms underlying the formation of the branched microvilli. For Andino, he and his team are developing mathematical models to see how well the organoid data correspond to real-world clinical data.

Andino is eager to see if other common respiratory viruses such as influenza or rhinovirus also need cilia to cross the mucus barrier to infect nasal epithelial cells and spread throughout the upper respiratory tract.

"It really opens a new field to understand this in this context, and we can be excited about it," he said. "If you understand that particular mechanism better, you might have better ways to intervene with respect to preventing spread of the virus." ■

REFERENCES

1. **Wu, C.T.** ***et al.*** **SARS-CoV-2 replication in airway epithelia requires motile cilia and microvillar reprogramming.** ***Cell*** **186, 112-130.e20 (2023).**
2. **Lee, I.T.** ***et al.*** **ACE2 localizes to the respiratory cilia and is not increased by ACE inhibitors or ARBs.** ***Nat Commun*** **11, 5453 (2020).**