



Here's a T cell with dendritic cells (long, tree-like shapes) attached, in the process of transinfection. Pitt researchers are exploring what happens when dendritic cells have less cholesterol. They think that might protect against transinfection.

# HIV'S HOW

THE SECRET SHORTCUT TO  
FAST AND FURIOUS INFECTION

BY HEATHER BOERNER

Somehow, about 1 in 300 people who contract HIV are able to live disease-free for decades without medication. In these nonprogressors, as they're called, the virus replicates so slowly that it never reaches the tipping point of full-blown AIDS infection. Researchers have puzzled over these rare cases for some 30 years, hoping to find some unique biological signature that might hold the key to a vaccine, to no avail—until now.

In the May issue of the journal *mBio*, a team led by the University of Pittsburgh's Charles Rinaldo—a PhD and chair of the Graduate School of Public Health's Department of Infectious Diseases and Microbiology, who also has an appointment in the Department of Pathology in the medical school—may have figured out at least part of the reason these people are able to keep the disease at bay.

Researchers have long suspected that HIV must be using some kind of shortcut to spread through the body so rapidly. Rinaldo found that this is indeed the case—and that non-progressors naturally shut down that shortcut, called transinfection. They're able to do this because the white blood cells they use as sort of a canal system (dendritic cells, which have long extensions) don't have enough cholesterol to allow the virus to penetrate and spread. "Cholesterol forms lipid rafts," says Rinaldo. Those rafts ferry HIV, carried by dendritic cells, to helper T cells, which are then infected with the virus. Without the lipid, the raft breaks down, and HIV stays put, replicating steadily but slowly at the site of the infection.

For a scientist who has studied HIV since the early 1980s, the results were stark.

"Lab results aren't usually all or nothing," says Rinaldo. "But this one was. We didn't believe it. We repeated it many times."

They sat on the results for several years until

they could figure out why the dendritic cells didn't transmit HIV and create the explosion of virus in T cells that progressors experience. The breakthrough came when a visiting professor shared the work he'd been doing on cholesterol and transinfection. Working with cells from uninfected people, he'd found that if you alter the cholesterol in the dendritic cells and then add HIV, transinfection stalls.

Then Rinaldo's team pulled blood samples from the non-progressors—this time, testing the cholesterol levels in their cells. Though non-progressors had normal levels of cholesterol in their T cells, their dendritic cells were deficient.

And if they added cholesterol to these deficient dendritic cells? Transinfection happened seamlessly, and the infection took the fast lane.

The samples came from men who were members of the longstanding Multicenter AIDS Cohort Study, or MACS, which Rinaldo started in the '80s. Most of the samples were of blood long ago infected with HIV. However, two of the eight non-progressors they studied had first enrolled in MACS before contracting HIV. Tests on the stored blood cells of those men from before they were infected showed dendritic cells with the same inability to transfect T cells. "This was the key finding in the whole study," Rinaldo says. "This is very likely a genetic trait. Our study was the first to show in a natural infection of HIV in humans that transinfection is significant."

The next steps: Find the biomarker for low-cholesterol dendritic

cells and recruit healthy people with the mutation for studies on how cholesterol and transinfection function both in HIV and in other diseases.

"We have to be careful about being overly confident—this virus never ceases to surprise me," Rinaldo says. "But these people's bodies are trying to tell us something. We have to listen." ■

## INSIDE TRACT

One way to head off HIV's downhill slide toward AIDS may start in the gut. That's what research funded by the National Institutes of Health and published in the June issue of *The Journal of Clinical Investigation* revealed.

"You see, HIV ravages the gut, causing a vicious cycle of inflammation, kicking up gut microbiota and sending it out into the rest of the body through damaged intestinal linings," explains Pitt's Ivona Pandrea, MD/PhD professor of pathology. "All this fuels HIV replication in the T cells, hastening the slide toward AIDS; it can also cause increased blood clotting, which leads to HIV comorbidities like heart disease.

"But if we can keep the microbiota where it belongs in the gut and calm the inflammatory response, maybe we can slow the progression of HIV and reduce the incidence of heart disease," she says. Pandrea did this with pigtailed macaques. Using sevelamer (a drug used in people with chronic kidney disease) to bind microbial lipopolysaccharide (a key component of the microbial wall) and prevent microbes from escaping the gut in a process called microbial translocation, Pandrea and her team found that they could reduce inflammation, decrease replication of the virus, and reduce coagulation levels.

"It's not a miraculous treatment for HIV," she says. "But we've directly proven the relationship between microbial translocation and immune activation. From a pathogenesis point of view, it is important." —HB



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# GINS OF THE FATHERS

ALCOHOL AND THE  
NEXT GENERATION

BY ALLA KATSNELSON

COURTESY JENNIFER BOMBERGER LAB

It's something pediatricians are taught to discuss with their young patients: Alcoholism runs in families, they counsel, so if yours has a strong history of this condition, you should be especially careful about drinking. But researchers' efforts to pin down specific genes that contribute to this heritability have largely come up short. "Nobody has found a smoking gun that says, *This is a gene that causes alcoholism*," says Gregg Homanics, a professor of anesthesiology at the University of Pittsburgh (with a PhD in animal science). He and Andrey Finegersh, an MD/PhD student in his lab, decided to try a slightly different tack. "We thought that maybe in alcoholics, drinking a lot would cause some changes in what controls the genes—and that is what gets passed down to the next generation," says Homanics. The findings from the resulting study were published in *PLOS ONE* in June.

The idea that parents' life experiences can have effects on their children's biology is not new. For example, studies show that famine in one generation tends to increase the rates of obesity and diabetes in subsequent ones. These effects are not caused by changes in the genes themselves, scientists think, but in chemical markings at specific spots atop DNA that reg-

ulate how genes are expressed—or epigenetics.

With regard to alcoholism, a flurry of studies two decades ago reported behavioral differences in the offspring of animals exposed to alcohol. But researchers back then did not yet have a good understanding of epigenetics and could not explain what they found. Now, scientists studying alcoholism are coming back for a closer look. It has long been known that addiction can influence how genes are expressed, and because addiction takes years to develop, heavy drinkers may be especially susceptible to racking up such modifications.

Homanics and Finegersh speculated that exposing mice to alcohol would make their offspring less sensitive to it and therefore more likely to imbibe, since that's what seems to be happening in humans. But to their surprise, they saw the opposite. They had male mice inhale alcohol vapor for five weeks, then bred the animals with females that had no exposure to the substance. The resulting pups grew up to be more sensitive to alcohol's effects on motor control and reduction of anxiety, not less, and were actually more likely to avoid it than were the control animals.

They also showed differences in epigenetic markings on a gene called *BDNF*, which has

been associated with drug-taking behavior; that change took place in an area of the brain called the ventral tegmentum, which is thought to be involved in addiction. Strangely, though, only male offspring, not female, were affected.

The researchers don't yet have a good explanation for what they found, but Homanics notes that researchers at the University of Pennsylvania reported very similar results in a study of cocaine published last year. One potential explanation, he says, is that this inherited disinterest evolved as a protective mechanism. "So if an animal is exposed to some toxin, for example, then [its] offspring may be less inclined to consume whatever has that toxin in it," he explains.

If that were the case, and if the result transferred to humans, then developing alcoholism would require somehow overriding such a mechanism.

But another explanation is much more prosaic. "We are not able to model all aspects of alcoholism in mice with just one or two tests," Homanics says. "So maybe we just picked the wrong test." (Their studies so far have measured alcohol's effect on anxiety levels and coordination, as well as what happens when the mice have unlimited access to the substance.) His group is continuing to investigate behavior and epigenetics of alcohol exposure with the mouse model and its offspring.

Homanics says, "What our study shows is that there is a lot we don't know about the effects of alcohol that we need to think about—how it might influence not just drinkers themselves but [also] the kids they are going to have." ■

Stubborn, stealthy, and dangerous bacteria biofilms (green) grow atop airway epithelial cells from a cystic fibrosis patient.

# STEALTH MODE

THE RED OCTOBER  
OF CYSTIC FIBROSIS  
BY LILY DAYTON

**P***seudomonas aeruginosa*—which is found in soil, mud puddles, and even the crevices of showerheads—isn't a problem for most healthy people. However, this opportunistic bacterium is quick to invade the airways of those with chronic lung diseases like cystic fibrosis (CF) or chronic obstructive pulmonary disease. By late adolescence, the lungs of 80 percent of CF patients are permanently colonized by *P. aeruginosa*. Some 80 to 95 percent of CF deaths result from respiratory failure from various lung infections. "The thought—[regarding] cystic fibrosis patients—is that it's this infection, and really a robust but ineffective immune response to it, that causes a lot of damage in the lungs," says Jennifer Bomberger, PhD assistant professor in the University of Pittsburgh Department of Microbiology and Molecular Genetics. In a series of papers throughout the past six years, Bomberger has uncovered mechanisms that may explain how *P. aeruginosa* pulls this off:

Essentially, by going into stealth mode.

For its studies of host-pathogen interactions, Bomberger's lab team uses a unique model, culturing airway epithelial cells that come straight from lungs that have been removed from UPMC transplant patients suf-

fering from chronic lung disease. (They also culture cells from donors with healthy lungs to use as controls.) The researchers then grow the cells together with *P. aeruginosa* on a plastic membrane, its underside bathed in medium and its topside exposed to air. The cells behave as though they were in the lung.

Using live-cell imaging, the team watches as *P. aeruginosa* produces colonies of bacterial biofilms—slimy, mushroom-shaped structures that are a hallmark of chronic lung infection—in the mucus layer that lines these epithelial cells. *P. aeruginosa* itself is highly resistant to antibiotics, and the biofilm colonies it forms create a physical barrier that is antibiotic resistant, as well.

Bomberger has shown how *P. aeruginosa* delivers numerous virulence factors across the mucus layer and into host cells. The bacteria release vesicles from their membranes, which fuse with certain membrane molecules of host cells. This way, *P. aeruginosa* avoids having direct contact with the host, Bomberger explains. At the same time, within biofilms, the bacteria change their gene expression to stop producing virulence factors, allowing them to fly under the radar of the host's immune system. Bacteria near the center of biofilms also drop to a lower metabolic state.

Like *Red October*—a fictitious nuclear-missile-armed submarine that stalks coastal waters undetected, thanks to a stealthy propulsion mechanism—*P. aeruginosa* evades the host's defenses as it attacks cells.

"We've shown using this model that we can't solubilize enough antibiotic to kill [the bacteria] when they grow like this," says Bomberger.

Her data also suggest that a co-occurring viral infection dramatically enhances the ability of *P. aeruginosa* to form biofilms. During a viral infection, the host's innate immune response plays a critical role in defending against the virus. But while the immune system is fighting one pathogen, it leaves an Achilles' heel that's vulnerable to secondary infection. In the majority of cases, *P. aeruginosa* takes hold in the lungs of patients soon after they contract a virus. Bomberger is trying to elucidate this process of co-infection to target the early stages of *P. aeruginosa* colonization. In addition, her lab is developing a biofilm-disrupting agent she hopes will prevent *P. aeruginosa* infection in CF patients. "If we can figure out a way to prevent or at least prolong the time until patients get this chronic infection, we can help their disease course," says Bomberger. ■

Editor's Note: Watch for more groundbreaking developments on CF in our next issue.