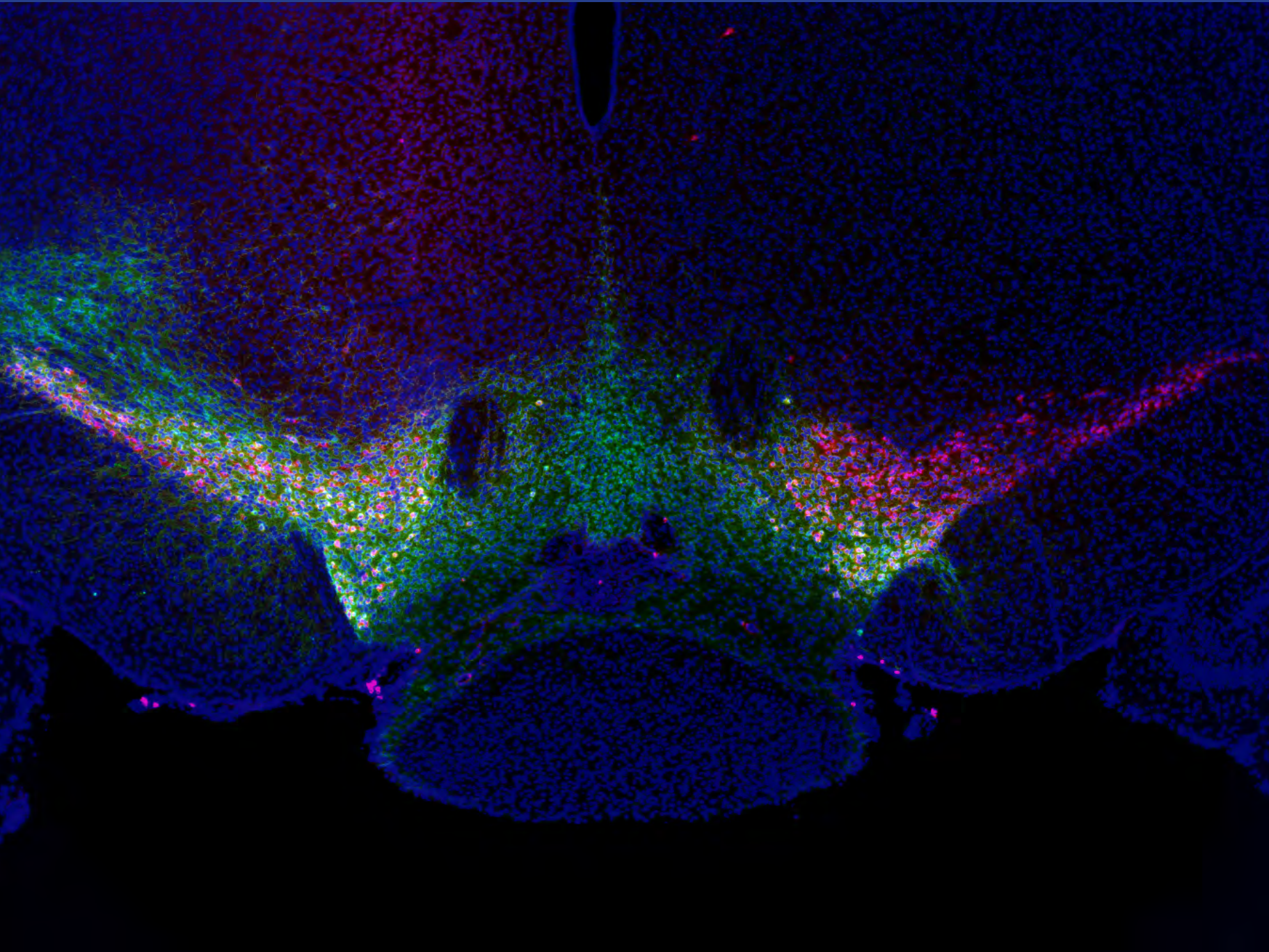


## INVESTIGATIONS

*Explorations and revelations taking place in the medical school*



McClung has made a direct correlation between the genes that control circadian rhythms and those in the mood-related circuits of the brain. Rodents with a mutation in their Clock gene—a model for mania—behave very similarly to rodents with impaired dopaminergic activity. Here, dopamine neurons, which are regulated by Clock, are shown in green. Shown in red is expression of an ion channel used to directly control dopaminergic activity.

# GOODNIGHT (MANIC) MOUSE

MODELING THE LINK BETWEEN MOOD  
DISORDERS AND THE CIRCADIAN CLOCK  
BY NICK CHARLES

It may sound surprising, but like humans, mice can exhibit both depressive and manic behavior. At times, the behavior seems to be linked to the “Clock gene.” Humans have the same gene, which is inextricably linked to the “24-hour rhythm in each of us,” says Colleen McClung, a PhD associate professor of psychiatry at the University of Pittsburgh School of Medicine.

It’s long been known that disturbances in the circadian clock—the master timekeeper of the body’s physiological processes—go hand in hand with mood disorders. (See our Summer 2012 issue cover story, “Sleeping’s Beauty.”)

“However, it wasn’t really understood why the genes involved in regulating circadian rhythms also affect mood,” says McClung. By using viral gene manipulation and pharmacological approaches, McClung has made a direct correlation between the genes that control circadian rhythms and those in the mood-related circuits in the brain. Her data suggest that new compounds that alter the circadian system might be developed into effective therapies.

Working in the Department of Psychiatry’s Translational Neuroscience Program, McClung studies how genes influence circuits of the brain that control mood. She does this by knocking out genes or by breeding mice with different kinds of transgenes—genes that overexpress functional or mutant proteins.

“We’ve used this method to create mice

that demonstrate behavioral responses similar to bipolar mania,” she says. “These particular mice have a mutation in the Clock gene (Clock Delta19) that renders this protein inactive.”

The method for creating this Clock mutant mouse was developed by other scientists in the 1990s; but it was McClung who characterized its mood-, reward-, and anxiety-related behaviors. Once she discovered that the mutant mice displayed the hallmarks of mania, such as hyperactivity, risk-taking behaviors, decreased anxiety, and lower levels of despair, she began a series of experiments that led to a Rising Star Translational Research Award from the International Mental Health Research Organization, bestowed in July 2012.

“The search for better treatment options

found that these medications restore healthy behavior in the mice.

The team also identified a group of enzymes known as histone deacetylases (HDACs) that may present better targets in bipolar disorder treatment. HDACs repress the expression of the dopamine regulator cholecystokinin (CCK), among other genes. McClung found that inhibiting HDACs actually counteracts the effects of Clock mutations.

With her Rising Star grant, she is now searching for the best HDAC to take aim at and the best agent to inhibit it.

“Although [lithium and valproate] have proven effective in treating bipolar disorder, their use has been shown to lead to liver and renal impairment, weight gain, nausea, and

**“It wasn’t really understood why the genes involved in regulating circadian rhythms also affect mood.”**

for bipolar disease has been difficult due to the lack of suitable animal models,” McClung says. “The models that we’re working with are appropriate for screening new compounds that might be effective in treating this chronic and debilitating disease.”

McClung’s team studied responses to stimuli in the Clock mutants compared to healthy mice, measuring behaviors consistent with mania. The Clock mutant mice were then given mood-stabilizing drugs (lithium or valproate) for periods of up to 14 days, and then the tests were performed again. The team

tremors,” she says.

Her hope is that, by inhibiting specific HDACs, it might be possible “to have the desired results of the drugs without the toxic effects.”

McClung has had several papers on her work with the Clock mouse published, as early as 2007 in *Proceedings of the National Academy of Sciences*. The most recent was October 2012 in the *European Journal of Neuroscience*. Her lab also has current grants from the National Institute of Mental Health and the National Institute of Drug Abuse. ■

# A GUTSY TURN

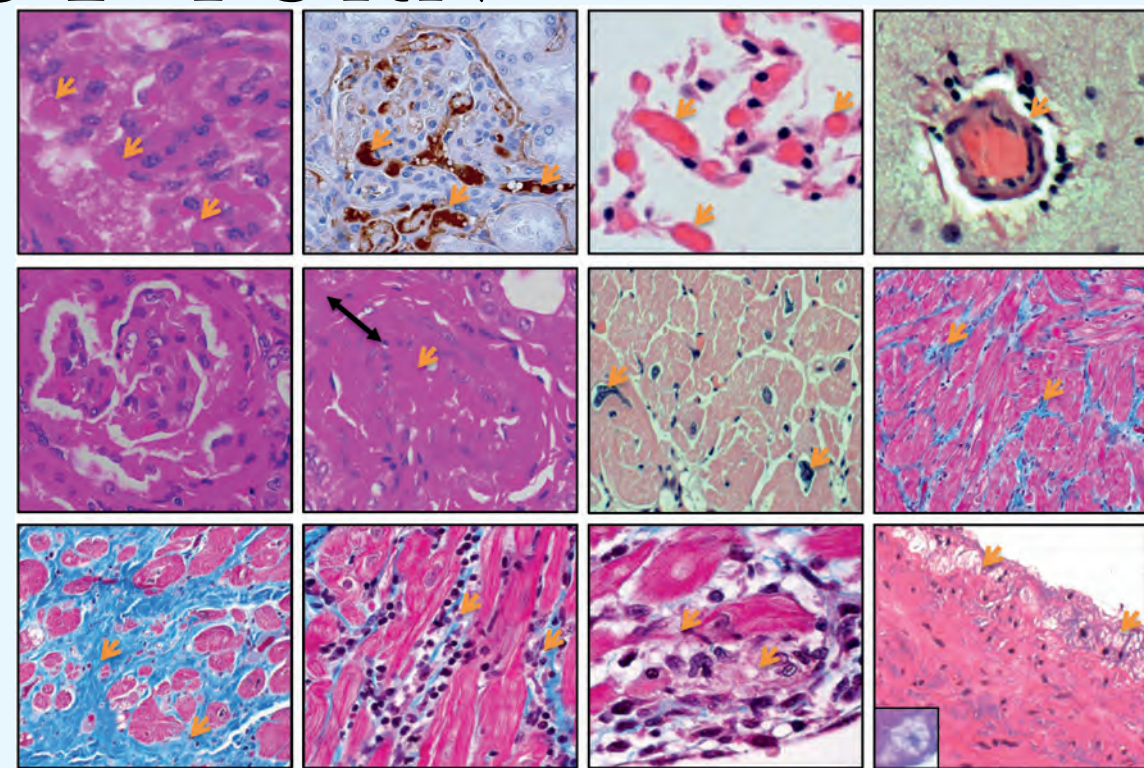
## A CULPRIT FOR EARLY CARDIOVASCULAR DEATH IN HIV PATIENTS

BY MELINDA  
WENNER MOYER

**I**t's a conundrum that has mystified virologists for years: Antiretroviral drugs do an excellent job of suppressing viral replication and opportunistic infections, yet HIV-infected patients still die young, frequently from heart problems. Some experts have blamed the drugs themselves; others have fingered unhealthy lifestyle choices common in HIV-infected patients, such as smoking. With so many confounding variables, it's difficult to tease out the true causes. But by studying how an HIV-like virus called SIV affects nonhuman primates, University of Pittsburgh pathologist Ivona Pandrea has found that the complications that afflict HIV patients stem not from drugs or cigarettes but from chronic immune activation—a finding that could have huge implications for HIV treatment.

More than a decade ago, while working in Gabon, Pandrea discovered that African green monkeys and mandrills can get SIV—simian immunodeficiency virus—without ever developing AIDS. Since then, Pandrea has been working to understand how these “non-progressives,” as she calls them, differ biologically from “progressive” primates, like pigtail macaques, which do develop AIDS. In a 2007 study, she reported that CD4+ immune cells in nonprogressive primates express low levels of CCR5, the receptor to which SIV attaches. The reason infection does not progress in these primates, she found, may be that the virus can't enter their target cells.

More recently, Pandrea has turned her attention to understanding how SIV infections unfold in these different primates. People with HIV typically have high incidences of cardiovascular problems like thrombosis and



high levels of immune activation and inflammation markers in their blood. In a study published in *Blood* in August 2012, Pandrea reported that progressive primates, including pigtail macaques, also have high levels of these markers and that they develop serious cardiovascular lesions, much as HIV-infected people do. Nonprogressives such as African green monkeys, however, do not have these markers or these symptoms. Considering that primates do not take antiretroviral drugs or smoke, she says, the findings suggest that the virus itself is the cause of the problems.

But how? Research by other scientists has shown that one of the symptoms of HIV infection is “leaky gut” syndrome: Because the intestines are home to many immune cells, early in infection HIV damages the gut mucosa, causing pieces of bacteria to “leak” into the bloodstream. Included in this harmful debris is lipopolysaccharide (LPS), a toxic component of some bacterial cell walls that, scientists speculate, causes inflammation and immune activation. When Pandrea injected nonprogressive African green monkeys with LPS for three weeks, she found that, sure enough, there was “an increase in the levels of immune activation and inflammation and coagulation in these animals,” she says.

Pandrea wondered whether leaky gut-induced immune activation and inflammation

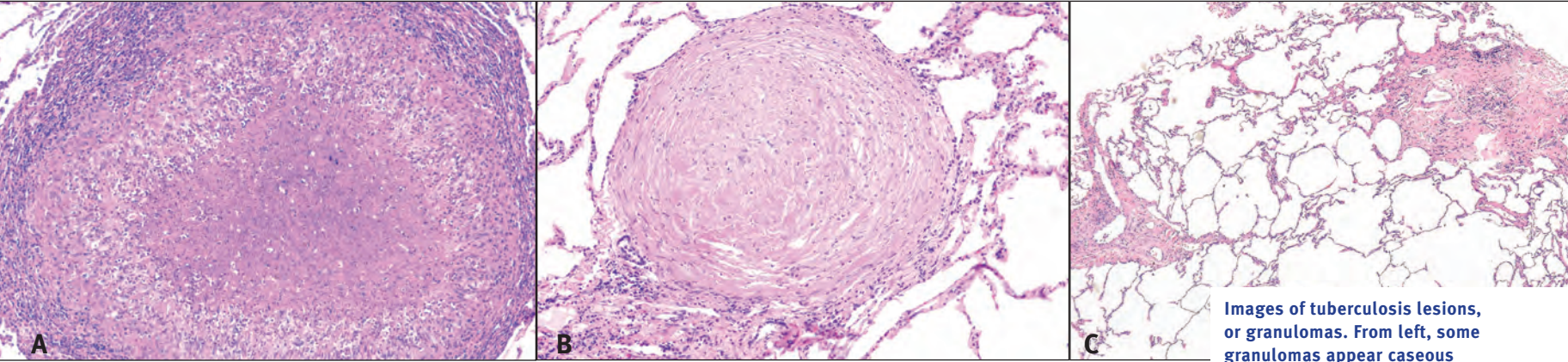
**Pigtailed macaques with SIV, the primate version of HIV, develop serious vascular problems. Here, arrows mark lesions in an infected primate's kidney, lung, brain, and heart, as well as in its aorta.**

could be a difference between avoiding AIDS and getting it. But perhaps it's not surprising that injecting toxic bits of bacteria would cause inflammation and an immune response. So, to place a final piece of the puzzle, Pandrea and her colleagues wanted to know whether by preventing inflammation and immune activation in progressive SIV-infected primates they might also be able to keep the animals healthy.

In an as-yet-unpublished study, they treated SIV-infected pigtail macaques with a combination of antibiotics and anti-inflammatory drugs, which also prevent leaky gut (also called microbial translocation). “The result was great,” she says. Markers of immune activation, inflammation, and microbial translocation dropped drastically.

Pandrea treated the primates early in their infections, before people are typically diagnosed with HIV, so she doesn't know whether the approach would be realistic for humans. Still, Pandrea is excited, and she notes that some of her collaborators are already testing similar treatments in clinical trials: “We identified a treatment that may be useful for HIV patients.” ■

# KILLING RESISTANT TB



Images of tuberculosis lesions, or granulomas. From left, some granulomas appear caseous (“cheese-like”) before medical intervention, then become fibrotic during successful drug treatment. Finally, when the bacteria are eliminated, scarring (interstitial fibrosis) remains.

## LOW-OXYGEN PLAYERS ARE A NEW TARGET | BY DANA YATES

**T**uberculosis is an intrinsically unpredictable disease. Spread through the air, TB germs cause an active infection in some people and an inactive one in others. And even among those with dormant TB, there is no way of knowing who will always breathe easy and who will end up with the disease (possibly infecting others). Findings derived from studies of monkeys by faculty members at the University of Pittsburgh suggest that reactivation of latent TB could be better prevented if a drug that is effective against bacteria in low-oxygen environments is added to the treatment regimen.

The results of the study by JoAnne Flynn, a PhD professor of microbiology and molecular genetics and an associate member of the Pitt Center for Vaccine Research, and Philana Ling Lin, an MD and assistant professor of infectious diseases, were published online in the Early Edition of *Proceedings of the National Academy of Sciences* last July.

In its active form, pulmonary TB causes people to cough up blood and mucus and experience night sweats, fatigue, fever, and weight loss. Without proper treatment, up to two-thirds of those infected will die of the disease. In fact, according to the World Health Organization, 1.4 million people died from TB in 2011. Among infectious agents,

it comes in second, after HIV/AIDS, as the greatest killer worldwide.

A person can become infected by inhaling only a few airborne droplets from a sneeze or cough of someone who has TB. Nevertheless, most people who come into contact with the bacterium *Mycobacterium tuberculosis* develop an asymptomatic, latent infection—one that cannot be transmitted to others. While the bacteria remain in the lungs, they are safely contained in a lesion, or granuloma.

For a small percentage of those with latent TB, however, the disease can stir at any time, and in the early stages of the disease, show no symptoms. In turn, each of these people, who number two billion worldwide, can infect up to 10 to 15 others through close contact throughout the course of a year.

Currently, active TB that is not resistant to antibiotics is treated with two months of the drugs isoniazid (INH), rifampin (RIF), pyrazinamide, and ethambutol, followed by four more months of INH and RIF. Latent TB, meanwhile, is treated with nine months of INH. In either scenario, the treatment isn't easy. INH, for example, can cause liver damage, and people tend to abandon the lengthy regimen before its conclusion.

“No one really knows why people have to be on the drugs for such a long time,” says

Flynn. “It’s just accepted that it’s due to the persistence of the bacteria.”

Hoping to find a better treatment, Flynn’s team targeted the center of the granulomas; these low-oxygen settings are filled with dead cells. Previous work by other researchers had proven that TB bacteria that can survive low-oxygen conditions are resistant to INH.

So, Flynn’s team speculated: Could TB bacteria be destroyed by a drug that specifically attacks nonreplicating bacteria in low-oxygen environments? In a project that was partly funded by the Bill and Melinda Gates Foundation, the researchers tested their idea using the antibiotic metronidazole (MTZ) in primate models. Ultimately, the team determined that two months of MTZ alone was just as effective as two months of INH and RIF at preventing reactivation of the infection. Moreover, adding MTZ to an INH and RIF regimen reduced the number and virulence of the bacteria in monkeys with active TB within two months.

One problem: Flynn notes that MTZ isn’t safe for humans if taken for an extended time. However, she is excited about what they’ve learned.

“Low-oxygen bacteria are indeed present in TB, and you can use a drug to treat them,” says Flynn. ■