

TB sanatoria stressed lots of fresh air.



A BADLY NEEDED NEW APPROACH FOR TB'S  
CENTURY-OLD TREATMENT | BY JENNY BLAIR

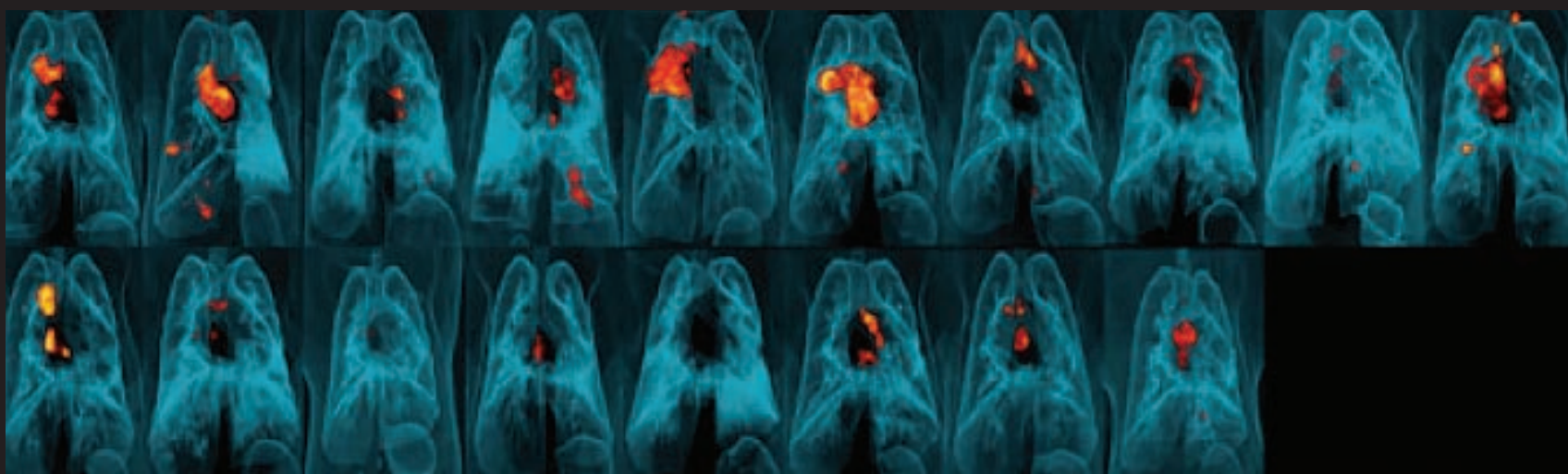
# ANOTHER SHOT FOR AN OLD VACCINE

Amid the crowded, tubercular cities of mid-19th-century Europe, the sanatorium movement was born. Rows of bedridden, anemic tuberculosis patients—their pallor giving the disease its nickname “white plague”—rested outdoors or on porches, breathing fresh air and eating hearty meals. Though a tuberculosis vaccine was introduced in 1921, it was only modestly effective, and sanatoria remained popular until the 1950s, when antibiotics rendered open-air treatment obsolete.

Not so the vaccine. At nearly a century old, conferring only partial, temporary protection, it remains the only one available today.

One-third of humanity is now infected with *Mycobacterium tuberculosis*, sometimes called tubercle bacilli. In 2018 alone, 10 million people contracted TB and 1.5 million died, many with a drug-resistant form of the disease.

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Researchers from NIAID and Pitt showed that just by changing the method of delivering the TB vaccine, they could ward off inflammation (red and yellow) greatly in a monkey model. The top row shows typical vaccination delivery—through the skin. The bottom row shows delivery through intravenous injection.

In an inspired move, teams from the National Institute of Allergy and Infectious Diseases (NIAID) and the University of Pittsburgh have made that old vaccine astoundingly effective in monkeys.

The culmination of a years-long collaboration between NIAID’s Robert Seder and Pitt’s JoAnne Flynn, a professor of microbiology and molecular genetics, the research involved trying several routes of vaccine delivery—not only the usual welt under the skin, but also directly into the lungs, and, crucially, by intravenous (IV) injection. Monkeys that received the IV vaccine were almost all protected from later infection by tuberculosis. The results appeared in *Nature*.

As it gradually became clear that the vaccine had worked to prevent infection, Flynn was astounded.

“You’re like, *Could this be possible—that there’s nothing there?*” Flynn recalls. “It was stunning.”

“We said, *How would the route affect immunity and protection?*” Seder says of their experimental design discussions. “Lo and behold, the IV worked magically.”

“No other TB vaccine has come close to showing the efficacy that this IV [version of the vaccine] has shown. It blew our socks off,” said coauthor Charles A. Scanga, Flynn’s project manager. “This vaccine really has the potential to make a huge impact on global public health.”

Moreover, the IV vaccine’s translation to human medicine is a decent bet, because a monkey’s reaction to tuberculosis is so similar to that of humans, says Thomas Smithgall,

chair of microbiology and molecular genetics.

“Getting a paper in *Nature* is a big deal, and I think it really speaks to the importance of the work and also the sophistication of the model,” Smithgall says. “We’re much closer to rhesus macaques than we are to mice. . . . People aren’t highly inbred strains of mice.”

Tuberculosis spreads when people inhale bacilli small enough to enter the alveoli of the lungs. Once lodged there, the bacteria enter large immune cells called macrophages and begin replicating. Infected macrophages signal other immune cells to gather around, where they coalesce to develop a nodule called a granuloma.

What happens next? It depends. The host organism usually mounts an effective cellular immune response within about 10 weeks, and in 90% to 95% of people, the bacilli are walled off and controlled in the granulomas—an asymptomatic state called latent tuberculosis, which requires no vaccination to achieve. Many people live their entire lives in a latent state, never knowing they have been infected by tuberculosis bacilli.

But latency is no guarantee of ongoing health. Up to 10% of people with latent TB later develop reactivation disease. Among people with HIV, that rate is much higher.

When defenses fail, the bacilli escape and the immune system’s attempts to destroy them begin destroying lung tissue. Bacilli can also set up infections in other parts of the body. Active TB kills half of untreated patients. Meanwhile, coughing spreads the bacilli to fresh hosts.

The only available vaccine is called

Bacille Calmette-Guérin (BCG). It is a weakened live strain of a related bacterium called *Mycobacterium bovis*, and 2020 marks its 99th year in clinical use. Most of those who receive it are newborns, and it can protect young children relatively well from severe forms of tuberculosis. But it does little to stop pulmonary TB in older children and adults, the groups most often responsible for spreading the disease. BCG’s protection wanes after about 15 years, and giving booster shots doesn’t work.

And yet, BCG is the best we have; and Flynn notes, “Most [investigational vaccines] haven’t given us any signal at all of being worthwhile.”

## MODELS AND VISUALS

Here’s how the Pitt side of the collaboration unfolded: During her postdoctoral fellowship at the Albert Einstein College of Medicine, Flynn worked with a mouse model of tuberculosis. But mice don’t make granulomas or get latent tuberculosis, both central features of human disease. And it takes up to half a million bacilli to infect mouse lungs, while in humans, 10 or 20 bacilli can suffice.

So Flynn spent two decades establishing that a monkey’s reaction to the disease closely resembles that of humans. When monkeys inhale active tubercle bacilli, about half develop latent tuberculosis. Of those, some reactivate. And they form granulomas. “[Flynn] was among the first to realize how important it was to use nonhuman primates as an appropriate model for human TB research,” Smithgall says.

“We built the model from the ground up. We had to make it up as we went along,” Flynn says.

To watch granulomas evolve over time, the team used a positron emission tomography-computed tomography (PET-CT) scanner.

In a PET scan, a researcher injects radiolabeled glucose, which is preferentially taken up by more metabolically active cells—such as the teeming inner cores of active granulomas. If a PET image is overlaid with a CT image taken at the same time, granulomas whose locations and sizes are visible on CT light up thanks to the radioactive PET probe.

“It allows you to follow not just the structure of granulomas over time, but their function, their metabolic activity over time, as well,” Scanga says.

The team acquired the scanner in 2007 with funding from the Bill and Melinda Gates Foundation. It was one of the nation’s first PET-CT scanners to be installed in a biosafety level 3 (BSL3) lab, according to Scanga.

Using the scanner, Flynn made a crucial discovery: Two granulomas in the same animal model can behave very differently. One might successfully contain the bacilli and control their replication, while the other may fail and allow the bacilli to enter nearby lymph nodes.

“It revolutionized how we do TB studies,” Scanga says.

Paul Duprex, director of Pitt’s Center for Vaccine Research and professor of microbiology and molecular genetics, compares the old approach—where researchers infect a model, then see how things turn out without having seen the disease unfold—to watching a movie with only a first and a last frame.

“Bioimaging allows them to piece together what the story is,” Duprex says.

(Flynn is now working closely with Duprex and others at the Center for Vaccine Research to image COVID-19 in the lungs of animal models.)

## AN UNUSUAL ROUTE

With monkeys that react as humans do to tuberculosis and imaging tools to provide unprecedented detail, Seder and Flynn were eager to test vaccines.

Seder, who is chief of cellular immunology at NIAID, suggested they break away from the usual intradermal method of vaccination and also test inhaled and IV routes.

He had good reason to think one of those routes could work. About a decade ago, he was part an effort to develop a malaria vaccine by

using an inactivated form of the parasite that causes the disease. Working with monkeys, Seder gave the investigational malaria vaccine subcutaneously—similar to a mosquito bite. That didn’t lead to immunity. So he asked his fellow to try the intravenous route instead, believing it might be a better way to distribute the vaccine throughout the body.

It was.

Measurable immune responses to the vaccine showed up in the monkeys’ blood, and large numbers of T cells crucial to warding off malaria appeared in the liver. In 2013, Seder led a study evaluating a clinical trial of an intravenous malaria vaccine. It generated outstanding protection against malaria and is now in clinical trials in Africa.

Eventually, the Pitt team gave monkeys BCG vaccines using one of the following techniques: the intradermal route, an inhaled route, a combination of the two, or through an IV. Six months after vaccination, the researchers exposed the monkeys to virulent airborne mycobacteria. Then they waited, scanning the lungs every four weeks.

With the inhaled vaccine, results were disappointing. At first, T cells swarmed into the airways of the lung. But within months, they were gone, offering no protection.

Unsurprisingly, the intradermal vaccine resulted in partial protection from infection. The results weren’t much different when that was combined with aerosol.

By stark contrast, in the IV group, nine out of 10 monkeys were clearly protected. Nine showed no signs of lung mycobacteria in the imaging scans. Six showed no lung granulomas.

When the researchers counted up viable bacilli in the lungs, the median number in the monkeys receiving the standard BCG vaccine was nearly 800,000. That went for those vaccinated by aerosol or a combination strategy, too.

But in the IV-vaccinated animals, the median was zero. In six, the researchers could find no evidence of tuberculosis in any body tissue. The monkeys had either promptly eliminated early infection or prevented it outright.

“We couldn’t culture any mycobacteria from them; IV BCG elicited sterilizing immunity,” Scanga says. “That was something that really hadn’t been seen before in a

TB developmental vaccine.”

Why did the IV route work so well?

The researchers suspect that, instead of stimulating antibodies the way most vaccines do, the IV method gets T cells involved.

To keep TB infection under control, immune T cells must mount a response, learn to ward off the bacilli and remain in the body and lung over the long-term.

The common intradermal BCG vaccine doesn’t provoke this response. But after IV vaccination, memory T cells took up positions in both the airways and the lung tissue—then, crucially, they stayed, poised to defend, throughout the six-month duration of the experiment.

## NEXT STEPS

There are hurdles to clear before the approach can be adopted for widespread human use. First, scientists will have to prove it is safe. Then there are practical problems. Many global health workers aren’t trained to perform IV injections. This vaccine also requires uninterrupted refrigeration—a tall order in parts of the world where electric power is intermittent or scarce.

Yet a target population of adolescents and adults—whose veins are easier to find than those of babies, and who are more cooperative—might ease vaccination, Seder says. If a safe, protective vaccine must be given intravenously, he says, “I do not believe [that route] is a deal breaker.”

Moreover, understanding exactly how the vaccine confers such robust protection may help researchers develop other ways of delivering its protective effect. “We’ve now provided the ultimate benchmark for high-level protection with a TB vaccine, and now we can understand what they call immune correlates and immune mechanisms,” Seder says. “It’s a gold mine scientifically.”

Duprex notes that the results will “reach into not just the TB world, but into other infection-biology worlds.”

For now, the researchers will be testing whether a lower dose of BCG offers the same level of protection. They’ll also work to gain a detailed look at what’s happening in the lungs, looking for clues to how the vaccine functions.

“Even now, every day,” says Flynn, “I learn something new about TB.” ■