evidence again—including new analyses of occupational studies, according to an EPA scientist—the agency has now upped dioxin's classification to "known" carcinogen. Although dioxin levels in the environment have been steadily falling since the 1970s, once ingested, exposure continues because the pollutant bioaccumulates in body fat.

Michael Gough, a retired biologist and dioxin expert, asserts that "there is no convincing evidence" that dioxin is 10 times more potent a carcinogen. Scientists who helped write the report counter that evidence of higher potency is solid and should not be surprising: Even in 1994, EPA had recognized that dioxin has a much longer half-life in humans than in rats. What's more, the International Agency for Research on Cancer recently classified dioxin as a known carcinogen as firmer evidence has emerged from studies of workers exposed to dioxins. But even one scientist who co-authored a key chapter of the new study cautioned against reading too much into EPA's characterization of a 1-in-100 risk. "That's at the very upper edge of body burden in the United States," says Chris Portier of the National Institute of Environmental Health Sciences.

Meanwhile, EPA officials point out that the report is not final; the agency intends to release a draft for public comment in June and send it for final scientific review in September. With this inauspicious debut, the report seems sure to attract a wide audience.

–JOCELYN KAISER

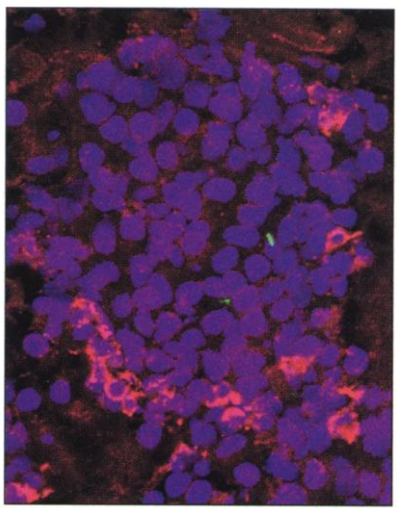
MICROBIOLOGY

New Clues to How the TB Bacillus Persists

The tuberculosis bacterium has been called the world's most effective pathogen. It kills an estimated 2 million people each year and lurks in one-third of the world's population. In most, the infection remains latent, but in some it explodes in disease years to decades later. Just how this organism manages to persist silently in the body in a kind of truce with the immune system has long been a mystery—one just now beginning to be solved.

Two groups, one led by Stanford University microbiologist Stanley Falkow and the other by microbiologist William Jacobs of Albert Einstein College of Medicine in New York City, have identified genes that may be required for persistent TB infection. If so, the proteins made by the genes could be good targets for new drugs against TB, which are badly needed because the pathogen is becoming resistant to current medications. The work is "clever and exciting," says infectious disease specialist William Bishai of Johns Hopkins School of Public Health in Baltimore. "If we understand how the organism adapts and persists, we'll be much better off in terms of drug development."

For their work, which is described on page 1436, the Stanford team used an animal TB model: the infection of frogs by *Mycobacterium marinum,* a close cousin of the TB bacillus, *M. tuberculosis*. The researchers chose *M. marinum* because it grows faster than *M. tuberculosis* and is not spread through the air. But it causes latent infections in frogs, similar to those in human TB. In particular, the animals develop a pathological hallmark of persistent TB infection: clusters of immune cells called granulomas where the bacteria live for years.



Turned on. The green glow marks *M. marinum* bacteria persisting in a frog granuloma.

CREDIT: RAMAKRISHNAN *ET AL.*

To identify the genes that allow *M. marinum* to persist, Falkow, with Stanford colleagues Lalita Ramakrishnan and Nancy Federspiel, attached the gene for a green fluorescent protein to random fragments of *M. marinum*'s genome and then inserted the fragments, carried on circular pieces of DNA called plasmids, into normal strains of the bacterium. That way, if a fragment contained a gene that became active, its bacterial host would glow green, and the gene could be identified by its location on a plasmid.

Using this technique, Ramakrishnan and her colleagues found a dozen genes that *M. marinum* turns on when living inside frog granulomas but not when growing on its own. Most of them have close counterparts in *M. tuberculosis,* whose genome was sequenced in 1998. Two in particular caught the researchers' interest, because they are members of a family of genes called PE-PGRS that comprises 5% to 10% of the *M. tuberculosis* genome. These genes are blueprints for bizarre-looking proteins that contain hundreds of glycines in a row and whose functions are unknown. Intrigued, the Stanford researchers knocked out one of the PE-PGRS genes in the frog bacterium.

When they put these mutants into macrophages, the main type of immune cell in granulomas, that were maintained in lab cultures, the bacteria didn't grow at all. And they grew only poorly in frog granulomas, with the number of bacteria in the frogs reduced to 1/50 that of wild-type infections. That suggests that at least some of these PE-PGRS family members play a role in persistence. "This is the only demonstration that these genes have any function," says Eric Rubin, a microbiologist at the Harvard School of Public Health in Boston.

Others aren't convinced that the Stanford group's finding casts any light on human TB, however. Ian Orme, a TB researcher at Colorado State University in Fort Collins, thinks the frog model is a poor one, in part because *M. marinum* is relatively harmless to mammals and must be injected in "monster doses" to produce signs of infection in frogs. "I'm not arguing that frogs are people," counters Falkow. "But *marinum* and *tuberculosis* have a common ancestor, and they share a common pathogenic apparatus."

One way to settle the argument would be to knock out the PE-PGRS gene in *M. tuberculosis* itself and see whether that affects the organism's virulence. Such an effort could be greatly facilitated by a method developed by the Einstein researchers in the course of their work, which appears in the April issue of *Molecular Cell.*

Jacobs also started with a safer organism: bacillus Calmette-Guérin (BCG), used in the TB vaccine. Two years ago, he and Einstein colleagues Michael Glickman and Jeffery Cox began randomly knocking out genes in BCG looking for mutants that failed to form cords, a phenomenon in which the microbes join in long ropelike structures in culture. Because such "cording" seems to correlate with high virulence, Jacobs reasoned that pinpointing genes needed for cording might lead him to virulence genes.

The researchers found that mutations in a BCG gene that makes an enzyme called cyclopropane synthase prevent the bacteria from forming cords. They then went on to inactivate the comparable gene in *M. tuberculosis.* In the past, producing such gene knockouts took 6 months, in part because it's very hard to get gene-disrupting DNA fragments into the TB genome. But the group used a rapid new technique co-developed by Einstein's Stoyan Bardarov, which uses a TB-infecting virus to insert the fragments, and so achieved its goal in just 3 weeks.

The mutant TB strains thus obtained also failed to form cords. What's more, they were less virulent. Control animals infected with the unaltered microbe all died from the infection after about 7 months. By contrast, the mutant bacteria grew rapidly in their hosts for a few weeks, but then began to die off slowly,

without killing any of the mice. These results, Jacobs says, show that the mutated enzyme is not only critical to the ability of *M. tuberculosis* to cause disease but also to its ability to persist. "This is the first persistence mutant ever isolated," claims Jacobs.

Others question whether the gene is involved in persistence, partly because the mutant strains did not rapidly disappear in the mice after the initial stage of infection; thus, knocking out this gene did not dramatically cripple the bacterium's ability to persist. "It's an interesting gene, but I wouldn't consider it the crux of persistence," says Bishai.

But nobody is questioning the impact of the new technology for creating TB mutants, which Harvard's Rubin hails as "a huge advance." Indeed, Jacobs says that in the past 6 months his team has created about four times as many TB mutants (32) than have been published to date.

Both groups have more work to do in figuring out what role the genes they have identified might play in TB virulence and persistence. But in any new knowledge, there is hope. Says microbiologist Michael Mahan of the University of California, Santa Barbara: "The payoff is huge when you really understand a disease." —INGRID WICKELGREN

BIOMEDICAL ETHICS

HHS Plans to Overhaul Clinical Research Rules

Health and Human Services (HHS) Secretary Donna Shalala announced last week that the government intends to issue new guidelines and regulations designed to protect human subjects who participate in clinical trials. Curiously missing from the announcement, however, was the final word on a long-anticipated reorganization of HHS's framework for monitoring patient safety in clinical research.

The department is planning to appoint a "czar" who will run a new office in HHS that will coordinate efforts by 17 agencies to protect human research subjects. According to several sources, the job is being offered to Greg Koski, an anesthesiologist and director of human research affairs at Massachusetts General Hospital in Boston. But a deal had apparently not been consummated in time for last week's announcement, which came on the eve of congressional hearings on the topic, and the department was left proclaiming a new policy but not the person who will implement it.

> **HHS needs "a world-class leader"... from "outside the bureaucracy."**
>
> —Donna Shalala

The announcement said HHS plans to ask Congress for civil penalties for lapses in obtaining informed consent from research subjects—up to $250,000 per clinical investigator and $1 million per institution. Both the National Institutes of Health (NIH) and the Food and Drug Administration are drawing up new guidelines on obtaining consent. HHS also plans an "aggressive effort" to train clinical investigators and members of Institutional Review Boards (IRBs) on the use of human subjects. And NIH intends to clarify its guidelines on conflict of interest to ensure that "any researcher's financial interest in a clinical trial [is] disclosed to potential participants."

As for the new czar, Shalala said earlier this month that the job will go to "someone who is experienced and is a world-class leader" but is from "outside the bureaucracy." That formula clearly ruled out one top candidate: Gary Ellis, the current director of the Office for Protection from Research Risks (OPRR), a small office at NIH that watches over institutions receiving federal money for clinical research. Ellis confirms that he will not go to HHS when the office moves but instead will be offered other employment at NIH. Under Ellis, OPRR experienced a sudden change of style. After being criticized in Congress in 1998 for not taking the initiative, OPRR came out with guns blazing over the past year and a half, shutting down half a dozen prestigious clinical research programs for noncompliance. Ellis's high-impact style may have cost him some support among university chiefs, observers say. But it also won praise. One leader in the field says Ellis "raised the public consciousness." He also got an endorsement from Representative Dennis Kucinch (D–OH), who said this month that Ellis's record was "the only bright spot" in a "dismal area" of federal oversight of human research subjects.

Koski—if he is the appointee—will clearly be wading into a contentious political job. An assistant professor who has spent the past 30 years at Harvard and Harvard Medical School conducting basic research, clinical medicine, and teaching, Koski declined comment.

One of the czar's first tasks will be to answer questions about federal monitoring of experiments such as the gene therapy trial in which an 18-year-old died last September (*Science*, 12 May, p. 951). The Senate public health subcommittee has scheduled a hearing on this case for 25 May. Meanwhile, a House Government Reform subcommittee, chaired by Representative

ScienceScope

Hot Air? When Congress ordered the federal government 4 years ago to sell off its massive helium gas stockpile by 2005, some scientists got a sinking feeling. They worried that the result could be shortages which would hamper a host of research-related technology efforts, from the development of fiber-optic cable to magnetic-resonance imaging systems. But a National Research Council report released this week says the end of the federal monopoly on the element is unlikely to affect users—in science or industry. Even so, the report calls for the government to explore new ways to locate supplies of the gas, improve storage systems, and search for substitutes.

Cash to Burn The wildfire that scorched the Los Alamos National Laboratory in New Mexico disrupted research and reduced some offices to rubble—but it may yield a hefty consolation prize from Congress. The Senate last week added $85 million to a defense spending bill to help the lab rise from the ashes that followed a controlled park-land burn that ran amok (above). The House is expected to follow suit.

CREDIT: AP PHOTO/THE NEW MEXICAN, S. POYNTER

The lab's 7000-person workforce is "almost completely" back on the job after 2 weeks off, says associate lab director Tom Meyer. And despite the destruction of dozens of employee's homes and about 30 chemistry offices in temporary trailers, officials say fears of harmful releases from stored waste have so far proven unfounded. Spectrometers and other equipment are still being checked for smoke damage.

Meanwhile, lab officials are making recovery plans, including how to spend $26 million for environmental restoration that is part of the defense bill. First on the agenda are measures to handle excess runoff from denuded watersheds, which could be hard hit by approaching "monsoons." Still, fire damage may pale in comparison to the wounds—in morale and hiring—inflicted by recent allegations of Chinese spying at the lab. Says physicist David Campbell of the University of Illinois, Urbana-Champaign, former head of the lab's Center for Nonlinear Studies: "The spy stuff was much more devastating."