Shaped like a cone: The configuration of 'virulence factors' that allow TB to invade the lungs

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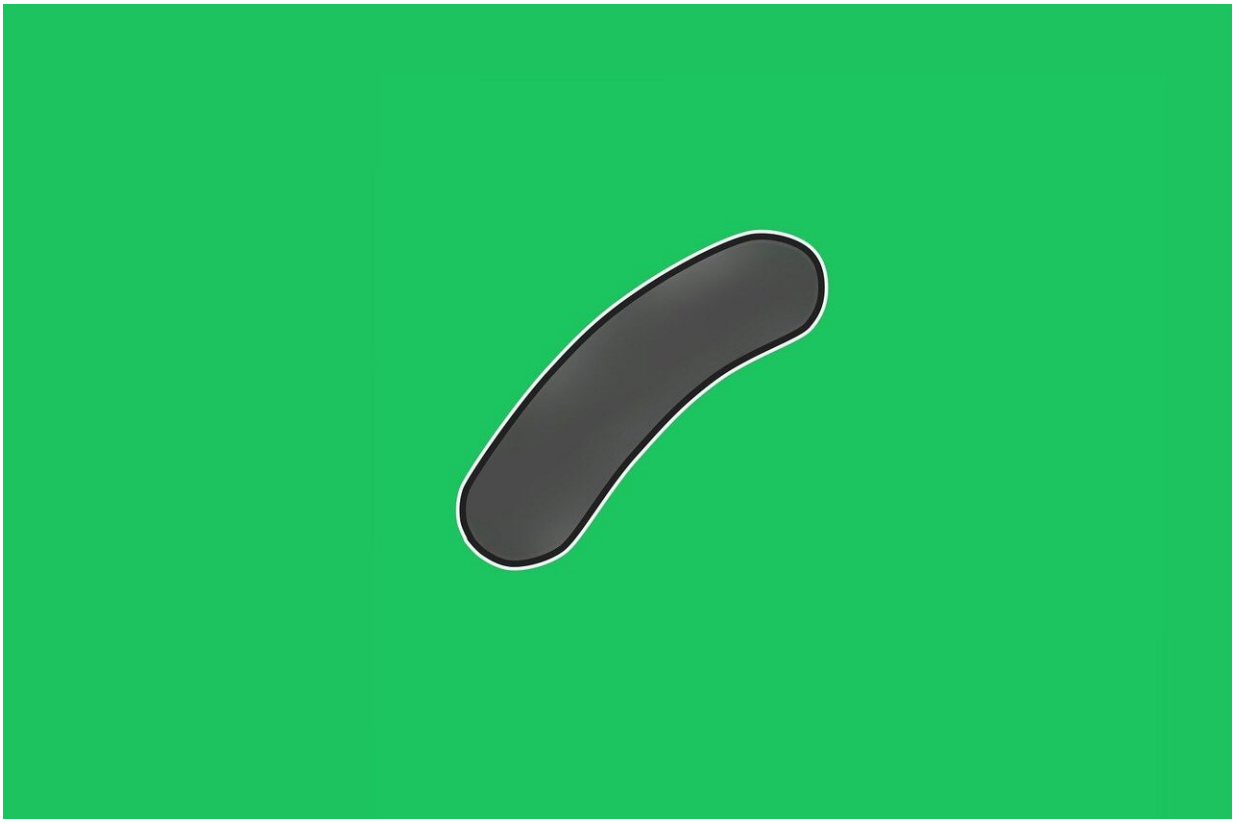

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The bacterial pathogen that causes tuberculosis is a master of deception, a king of clever tricks—an enemy agent that not only infiltrates but can

become a long-term stowaway in patients' lungs.

Mycobacterium tuberculosis not only eludes immune system surveillance, it boldly invades lung macrophages, the very cells that are supposed to annihilate it. Worse, the bacteria not only invade—they can hide indefinitely in macrophages, normally the brutes of the immune system, capable of engulfing and transforming infiltrators into harmless debris. M. tuberculosis flips the script into a completely different story.

Now, scientists in France have for the first time unveiled the devastating first step in the infection process, which is led by potent virulence factors known as phthiocerol dimycocerosate, or more simply as DIM. These complex lipids promote the entry of M. tuberculosis into lung macrophages.

Although scientists have known about these lipids for decades, it has taken until now to reveal the molecular maneuvers that allow these lipids to gain entry into macrophages—and to learn how their shape aids their ability to break and enter undetected.

"DIM lipids have been known as key virulence factors for more than 40 years, and our work showed for the first time a molecular mechanism of action for these lipids. Indeed, we show that their conical shape is linked to the ability of the bacteria to invade," Dr. Jacques Augenstreich told Medical Xpress. He is the first author on a major report that peels away much of the mystery underlying an ancient microbe's deepest secrets.

Published in the *Proceedings of the National Academies of Sciences*, Augenstreich and colleagues explain how they combined structural biology, computer modeling and biophysical methodologies to reveal how DIM's action on macrophage membranes leads to the critical first steps in tuberculosis infection.

Augenstreich, now with the University of Maryland, was part of a team that included Drs. Alain Milon, Catherine Astarie-Dequeker, and Matthieu Chavent of the Institut de Pharmacologie et de Biologie Structurale in Toulouse, France.

At the core of their research is a discovery that explains why the bacteria are able to hide in macrophages. Their research also reveals how DIM virulence factors play a key role.

"These lipids have a particularity to be almost completely hydrophobic, with no polar head, or epitopes that could eventually be recognized by the immune system," said Augenstreich, noting that the bacteria are able to elude surveillance and detection. "The fact that DIM lipids also localize within the macrophages' membranes, in their hydrophobic core, may hide them from the macrophages."

Tuberculosis (TB) is spread through the air by coughing, sneezing, spitting, and even casual conversation at close range. Infection is caused by a hardy microbe that can survive up to six months on surfaces, provided that the site is free of sun exposure or artificially produced ultraviolet light.

The pathogen is technically known as a rod-shaped bacillus—a non-motile, non-spore-forming obligate aerobic bacterium.

The World Health Organization estimates that 10 million people are infected with TB annually and 1.6 million die of it, making tuberculosis a leading cause of infectious disease mortality. Globally, more people die of TB than HIV, influenza and measles combined. The majority of cases occur in resource-poor regions of the world where drug-resistant strains have become frighteningly prevalent. A new drug, the first for TB in more than 40 years, was approved by the U.S. Food and Drug Administration in August. That medication, Pretomanid, is taken in

tablet form and designed to be used in combination with two other drugs. The regimen is designed specifically for highly drug resistant types of TB.

For people with non-resistant forms of the disease, treatment with other combinations of antibiotics is successful when patients adhere to the lengthy regimen, which can last six to nine months. Treatment is usually conducted under supervision—directly observed treatment (DOTS)—because of the high potential for some patients to shrug off compliance. Treatment success rates under DOTS are above 82 percent, according the World Health Organization.

But TB is a complicated disease for myriad reasons. Some patients have latent infections, disease that is marked by live bacteria that remains sequestered in macrophages. While in a latent state, an infected individual cannot pass along the disease. However, a latent infection can become active, and even more complex still, some patients can have latent and active disease at the same time.

Researchers in France, meanwhile, were able to zero in on the critical molecular steps in the infection process by relying on a variety of sophisticated tools.

"For this study, we used a multidisciplinary approach, from the atomistic level to the cellular level," Auguenstreich explained. "The shape of DIM was first investigated using in silico multiscale molecular modeling, combined with nuclear magnetic resonance that allowed us to show that DIM adopted a conical shape. Then these properties were explored in the context of macrophage infection."

In silico multiscale molecular modeling refers to computer modeling, which has become an indispensable tool in molecular biology, experts say.

Although DIM lipids are critical virulence factors, M. tuberculosis has an abundance of others, which explains why the infection has persisted as a leading cause of death for many millennia.

"The bacteria can secrete a broad range of proteins that can manipulate the immune response, and this feature is actually quite common for bacterial pathogens. It also produces numerous lipids that are unique to pathogenic mycobacteria," Augenstreich said.

Historically, TB has held sway as a formidable infectious disease for as long as humans have been on Earth. Evidence of the disease has been described in 6,000-year-old Egyptian mummies. Researchers have found South American mummies dating back more than 1000 with the telltale signs of TB, which in the worst of cases can spread beyond the lungs to become a systemic disease. Scientists now believe the disease had multiple sites of origin worldwide.

Augenstreich said there is still more to be learned about the infectious agent. And in terms of other virulence factors, he said, "Their role in the virulence of M. tuberculosis is not always understood. Their impact on macrophage membrane structure still needs to be more thoroughly investigated."

More information: Jacques Augenstreich et al. The conical shape of DIM lipids promotes Mycobacterium tuberculosis infection of macrophages, *Proceedings of the National Academy of Sciences* (2019). DOI: 10.1073/pnas.1910368116