Scientists invent new way to disarm malaria parasite

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A novel technique to "tame" the malaria parasite, by forcing it to depend on an external supply of a vital chemical, has been developed by researchers at the Stanford University School of Medicine and the University of California-San Francisco. The scientists have, in effect, created a domesticated strain of *Plasmodium* — the one-celled parasite that causes malaria — that would no longer cause this dreaded disease.

Their findings not only make it possible to grow large volumes of this modified parasite, but also reveal how the parasite's very survival turns on the production of one chemical — isopentenyl pyrophosphate, or IPP. These developments could help to speed up drug development and provide the basis for the first effective vaccine against malaria.

The study, which will be published online Aug. 30 in *PLoS Biology*, was conducted by Ellen Yeh, MD, PhD, an instructor in Stanford's Department of Pathology, and UCSF professor of biochemistry and biophysics and Howard Hughes Medical Institute investigator Joseph DeRisi, PhD.

At the heart of the paper is a discovery by Yeh and DeRisi: The scientists identified IPP as absolutely essential to the malaria parasite's viability during the stage when it invades blood cells. Normally, *Plasmodium*'s IPP supply is manufactured in a unique structure within the parasite, called the apicoplast. IPP is pivotal to *Plasmodium*'s survival, but the researchers showed that during its blood-infecting stage, the parasite can live without its apicoplast — as long it continues to get

IPP from another source.

Malaria is one of the Earth's most notorious scourges, accounting for more than 250 million new cases each year, mostly in Africa but also in Southeast Asia, India and Latin America. It is transferred to humans via a mosquito bite, during which one-celled parasites of the genus *Plasmodium* are injected into the bloodstream. The resulting infection causes some 1 million deaths annually, largely among children under the age of 5.

At present, no effective malaria vaccines exist. What's more, *Plasmodium* strains usually develop resistance to drugs that have been approved to combat the disease. The World Health Organization, for instance, currently recommends artemesinin in combination with other, older anti-malarials for combating *Plasmodium* falciparum, the deadliest and most widespread form of the malaria parasite. But while that drug is still believed to be effective, reports of resistance are starting to emerge, said Yeh.

"If resistance becomes widespread, we're in big trouble, because there's little else in the pipeline that's not based on artemesinin," Yeh said.

Malaria is particular crafty and pernicious, changing its outward features like a master criminal undergoing serial plastic surgeries to evade detection. Injected by a mosquito into a person's bloodstream, the organism holes up for a while in the liver, producing no symptoms. After spending some time there, it changes its form and heads for the bloodstream. There, it invades red blood cells, feasting on them and reproducing, only to break them open, swim out into the bloodstream and find new red blood cells to infect. It is in the blood stage that *Plasmodium* causes visible symptoms. The cycle of red-blood-cell reinfection continues, producing periodic waves of fever, chills, fatigue and sweats.

At each stage — in the mosquito, in the liver and in the blood — the parasite assumes a new form with different surface features, thus presenting a different face to the immune system, stumping that molecular detective squad's efforts to mount a counterattack.

But down inside, *Plasmodium* remains the same old pathogen. Although one-celled like a bacterium, it's a protozoan — much bigger and more complexly organized than bacteria. *Plasmodium*, unlike bacteria, contains many of the same intracellular specialized structures and compartments our own cells do: for example, a cell nucleus, and the peewee power-packs called mitochondria.

However, all *Plasmodium* parasites also sport a cryptic internal feature our cells lack: the apicoplast. Although it's clear that the parasite can't live without it, the apicoplast's role has remained a mystery over the 15 years since its discovery. Yeh and DeRisi figured out that while the apicoplast manufactures many products, only its production of IPP is essential for the parasite's survival during its blood stage.

To achieve this insight, the researchers dosed blood-rich cultures of the parasite with antibiotics. It is known that antibiotics cause *Plasmodium* to cast off its apicoplast. This eventually causes the organism's death, but too slowly for antibiotics to be of any significant therapeutic use by themselves (although they can be used as prophylactics or in combination with other, faster-acting drugs).

Yeh and DeRisi found that if they added antibiotics to the culture medium along with a single substance, IPP, the apicoplast-lacking parasites could thrive in culture. "This showed that IPP is the only product *Plasmodium* really needs from its apicoplast during its blood stage," Yeh said.

This first-ever cultivation of an apicoplast-free malaria parasite promises

to advance efforts to come up with new drug and vaccine leads.

Mammals, too, need IPP as a starter material for myriad end products (one of the most well known is cholesterol), but they make IPP in a completely different way, using another set of enzymes. So a drug that knocks out this capacity in a *Plasmodium* apicoplast could wipe out the parasite without necessarily having any deleterious effect on human cells' ability to make this important precursor substance.

"This potential pathway for killing parasites without interfering with human cells is the reason the apicoplast has been a major focus for drug development," Yeh said. "Now we have a way to specifically look for drugs that target its function and discover a whole new class of desperately needed anti-malarials."

The researchers' discovery of how to grow apicoplast-lacking parasites in volume — feed antibiotics supplemented with IPP to cultured *Plasmodium* — may also aid vaccine development. "You could, in principle, safely inoculate patients with this version of *Plasmodium* because it can't survive for long in the human body," said Yeh. It's still alive, and it's the spitting image of the virulent version, so it can train the immune system and therefore stimulate good protection. "But without a supply of IPP, it's only good for one more cycle of reproduction. It soon dies off."

Moreover, she said, you can use the same in-vitro growth method on any *Plasmodium* strain that happens to be circulating in human populations, and quickly, cheaply and easily cause it to shed its apicoplast, thereby generating an attenuated, or less virulent, version of the strain. Other methods of creating attenuated strains, such as genetically modifying them, are slow, costly and difficult. Moreover, an apicoplast-deficient parasite is unable to mutate back to restored viability, because it has lost its entire seven-enzyme assembly line for manufacturing IPP.

Yeh cautions that it will be many years before this research gets to the clinical stage. "But we're closer now than we were before," she said.

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