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## Production of Sticky Proteins May Explain How Malaria Evades the Immune System

Researchers have discovered a sticky new strategy that the malaria parasite, *Plasmodium falciparum*, uses to make sure it evades the human immune system and thrives inside the body.

Near the end of its complex life cycle, the malaria parasite invades human red blood cells and produces a sticky protein called *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1). This protein migrates to the surface of the infected blood cell, where it grabs onto the inner wall of blood vessels. The result: Sick blood cells that clump together, which provides a safe haven for *Plasmodium* parasite to replicate out of control but also restricts blood flow in its human host.

Malaria researchers have long thought that the parasite produces just a single variation of the PfEMP1 protein at any given time. But new research from Howard Hughes Medical Institute international research scholar Anja T. R. Jensen has overturned that long held view. Jensen's research shows that *Plasmodium falciparum*—the deadliest of the handful of known malaria parasites—can simultaneously produce two different PfEMP1 proteins. Jensen and her colleagues found that this doubling-up makes infected red blood cells stickier, a fact that may complicate the development of new malaria drugs and vaccines. The research is published in the September 2010 issue of the journal *PLoS Pathogens*.

Sticking to the inside of blood vessels “is how the parasite avoids the immune system,” says Jensen, who works in the Center for Medical Parasitology at the University of Copenhagen in Denmark. If the cells weren't sticky, they would circulate to the spleen, a hotbed of immune activity, and be destroyed.

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Jensen began studying PfEMP1 proteins to understand if variations in the protein might correlate with clinical symptoms. She hypothesized that some versions of PfEMP1—malaria parasites can produce some 60 variations of the protein—might cause the most severe symptoms. If Jensen could identify a variation associated with particularly severe disease, she reasoned that it would make a good target for vaccine development.

"We believe there are a subset of these proteins that are being mainly expressed in young children who are infected, and that those are associated with severe disease," Jensen says. With their immature immune systems, children are hit particularly hard by malaria, accounting for most of the million or so annual worldwide malaria deaths. Some 300 million people get infected with malaria each year, mostly in the developing world.

However, while isolating the RNA of *Plasmodium*-infected red blood cells, Jensen and her colleagues saw something unexpected. Instead of retrieving RNA for just a single variant of PfEMP1, the team pulled out RNA from several different PfEMP1 variants. But because the team was working with a population of infected red blood cells, at first they thought different cells might be producing different PfEMP1 proteins. "That puzzled us for a while," Jensen says.

They decided to delve deeper by first isolating infected red blood cells carrying a particular form of PfEMP1. Jensen's team isolated these cells by using antibodies to the form of PfEMP1 they were studying. And, in fact, the team used two different antibodies, each of which attach to a particular form of PfEMP1. Jensen expected each red blood cell to attract just one of the two antibodies.

But when the team examined the cells using confocal microscopy and flow cytometry, they saw that some of the infected blood cells displayed both antibodies. "We got a little lucky that some of the infected cells made two forms of PfEMP1 that we happened to have antibodies for," Jensen says.

To confirm the results, the team used a technique that fluorescently tags genes as they're being transcribed from DNA to protein. They made a red tag for one variation of the gene that codes for the PfEMP1 seen on the cells' surface, and a green tag for the second variation. If the parasite inside the cells were producing just one variation at a time, a red or green spot would

glow inside each cell. Instead, Jensen saw a yellow spot – meaning that the cells were producing both two variations simultaneously. “This was very unexpected,” Jensen says.

Next, Jensen’s team tested the stickiness of the red blood cells that produced either one or two variations of PfEMP1. The cells that made just a single PfEMP1 bound to one of two receptors found on the inside of blood vessels, called ICAM1 and PECAM1. But the cells that made two PfEMP1 variations could attach to both receptors, making them stickier than usual.

“This could be something that happens during the very first infection in an individual,” says Jensen, who plans to repeat the experiments with parasites isolated from malaria-infected patients. “Those individuals—mostly children—don’t have any immunity to malaria. The parasite needs to replicate. To avoid being removed by the spleen, it has to stick somewhere in the body. It could be a benefit to the parasite to express more than one PfEMP1 protein and to stick to more than one receptor.”