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Gene Expands Malaria's Invasion Options

The malaria parasite *Plasmodium falciparum* uses different pathways to invade red blood cells, evading the body's immune system and complicating efforts to create effective vaccines against the disease. A research team led by Australia's Alan F. Cowman, an international research scholar with the Howard Hughes Medical Institute, has identified a gene that the parasite uses to switch back and forth between invasion pathways.

Researchers from the Scripps Research Institute in La Jolla, California, and the Genomics Institute of the Novartis Research Foundation in San Diego contributed to the work, which was published in the August 26, 2005, issue of *Science*.

P. falciparum causes the most lethal form of malaria, which results in one million deaths a year worldwide.

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Some *P. falciparum* strains invade red blood cells via protein receptors on the surface that contain a sugar known as sialic acid. If scientists treat blood cells with an enzyme to remove sialic acid, the parasite can no longer invade. Other strains - including one called W2mef - can invade using the sialic acid receptors, but also have the ability to switch to other pathways if necessary.

"It's a bit like someone trying to get into a house with different doors," says Cowman of The Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia, and the study's senior author. "When you remove sialic acid, you block half the doors. W2mef normally goes in through the receptors with sialic acid, but it can switch - so it has two methods of entry."

To investigate how the parasite manages to switch to an alternative mode of blood cell invasion, Cowman and colleagues produced lines of the W2mef parasite that used sialic acid for invasion, and lines that could invade without it. Then they compared the differences in gene activity between the two types and identified two genes that warranted further study.

The team found only two genes whose activity differed between parasites that used sialic acid and those that did not. The first of these was a gene known as *P. falciparum* reticulocyte-binding like homolog 4 (*PfRh4*) that's similar to other genes known to play a role in the invasion of red blood cells by *P. falciparum* and related parasites. The second gene, *EBA165*, did not appear to produce a functional protein, and the scientists suspect it had been activated only because it was physically adjacent to *PfRh4*. Using a second, more quantitative approach, the team found that the two genes were 60- to-80 times more active in the sialic acid-independent parasites than in those that needed the sugar for cell entry.

These results suggested that activation of the *PfRh4* gene was required for the parasite to make the switch to sialic acid-independent invasion. Indeed, the team was able to find PfRh4 protein in sialic acid-independent parasites, but not in the sialic acid-dependent lines. And when the group constructed parasites in which the *PfRh4* gene was disrupted, they found that those parasites would not grow in the absence of sialic acid, although they grew normally on cells with the sugar - further suggesting that activation of the *PfRh4* gene is required for switching from sialic acid-dependent to -independent invasion.

“Activation of *PfRh4* represents a previously unknown mechanism to switch invasion pathways and provides *P. falciparum* with exquisite adaptability in the face of receptor changes and immune system responses,” the team concluded.

The results have important implications for the design of anti-malaria vaccines. The molecule on the parasite that binds to sialic acid receptors on host cells is considered a target in anti-malaria medications, but Cowman notes that if only that gene is blocked, some parasites can still use *PfRh4* to switch to other means of entry. “If both genes are disrupted, it blocks both ways of getting in,” he says.