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Blocking Enzyme Imprisons Malaria Parasites

Researchers have found a way to prevent infectious malaria particles from bursting out of their protective sacs by blocking the activity of a protein-snipping enzyme, called a protease. The research suggests that it might be possible to treat malaria infection with protease inhibitors to keep the infectious particles imprisoned until they deteriorate.

In an article published in the December 12, 2000, issue of *Proceedings of the National Academy of Sciences*, Howard Hughes Medical Institute investigator Daniel E. Goldberg and colleagues at Washington University School of Medicine reported that their experiments illuminate a little-understood but important infective mechanism used by malaria parasites.

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— Daniel E. Goldberg

Malaria afflicts as many as 500 million people worldwide and is responsible for the death of two million children each year. Yet, says Goldberg, remarkably little is known about the process by which malaria parasites, in the form of merozoites, exit from red blood cells, which are the initial sites of infection. "It was known, for example, that some proteases may be involved and that the merozoites come out in a concerted fashion. But that was about it," Goldberg said.

Researchers knew that when malaria parasites infect a red blood cell, they envelope themselves in a portion of the blood cell membrane, which forms a protective sac called the parasitophorous vacuolar membrane (PVM.) However, it was not known what, if any, role the PVM played once the malaria parasite had reproduced to form clusters of infectious merozoites that exit from the cell.

In their experiments, Goldberg and his colleagues treated cultures of red blood cells infected with malaria parasites with a drug called E64 that specifically blocks the activity of certain proteases.

Detailed studies of the protease-inhibitor-treated cultures revealed that clusters of merozoites are encapsulated in transparent membranes following treatment with E64. To determine the origin of these membranes, the scientists treated the cultures with fluorescent-tagged antibodies that attached to blood cell proteins or to PVM proteins. These studies revealed that the membranes that enclosed the merozoite clusters came from the PVM.

"Thus, we concluded that this protease inhibitor treatment seemed to block the parasites at a particular stage in the exit process," said Goldberg. "In the E64-treated cultures, we found that the merozoites could still get out of the red blood cell, but they couldn't get out of the sacs. This told us that there are two steps in the process by which these parasites exit the host red blood cell, and that the second step requires a protease," he said.

The researchers conducted additional experiments to determine whether the imprisoned merozoites were still viable—an important distinction if the discovery is to be used for further research, said Goldberg. They found that once the protease inhibitor was removed from the culture and the merozoites were allowed to infect red blood cells, the malaria particles showed the same ability to infect as untreated merozoites. Goldberg and his colleagues also isolated the merozoite PVMs themselves and showed that the PVMs also yielded viable, infectious merozoites.

"These findings mean that use of protease inhibitors to prevent rupture of PVMs provides us with an excellent technique for isolating large numbers of infectious merozoites to study the process of invasion, the merozoites themselves, and the membrane around the parasites," said Goldberg.

He also emphasized the clinical potential of using protease inhibitors as a treatment for malaria. "Now that we've defined a specific and important role for one or more cysteine proteases, we can begin the process of identifying the proteases involved in the rupture process," he said. "This identification offers an excellent opportunity to develop specific inhibitors that would block the exit process and prevent the parasites from getting out of their sacs and multiplying." Protease inhibitors have already proven clinically useful in treating HIV infection, Goldberg pointed out, and several other protease inhibitors are showing promise for other clinical applications.