Study of malaria parasite in patient blood finds distinct physiological states

November 28 2007

The malaria parasite has been studied for decades, but surprisingly, little is known about how it behaves in humans to cause disease. In a groundbreaking study published November 28 in the advance online edition of *Nature*, an international research team has for the first time measured which of the parasite's genes are turned on or off during actual infection in humans, not in cell cultures, unearthing surprising behaviors and opening a window on the most critical aspects of parasite biology.

That insight springs from the genomic analysis of parasites in their natural state, derived directly from patients residing in Senegal, and also from the researchers' use of innovative computational approaches to interpret their results. These computational methods helped to identify three distinct biological states of the malaria parasite: an active growth-based state, a starvation response and an environmental stress response, presumably related to the body's inflammatory response to the parasite. This physiological diversity was previously unknown and may help explain the widely varying course of the disease in different patients, from mild, flu-like illness to coma and even death.

"For the first time, we have glimpsed the biology of the malaria parasite in one of its most important environments -- humans," said co-senior author Aviv Regev, a core member of the Broad Institute of MIT and Harvard and an assistant professor of biology at MIT. "Our unique computational approach holds promise not only for understanding the malaria pathogen, but likely other important microbes as well."

"This work illustrates the true power that comes from developing the right computational methods and applying them to important biomedical problems," said co-senior author Jill Mesirov, director of Computational Biology and Bioinformatics at the Broad Institute of MIT and Harvard. "Even more importantly, it reflects scientific research at its best -- a global effort that brings together clinicians and researchers with diverse expertise, working directly with patients in areas hardest hit by disease."

In its natural state, the malaria parasite, Plasmodium falciparum, leads a complicated life. It proceeds through a series of distinct developmental stages both in humans and in mosquitoes, the main vector for disease transmission. Malaria researchers typically circumvent this complexity by studying the parasite in cultured cells. Yet in this artificial setting, few differences have been found in the genes that are turned on or off in various strains of P. falciparum. That uniformity is surprising, because it fails to explain the drastically different courses experienced by malaria patients.

To explore the basis for these differences, first author Johanna Daily, an infectious disease physician at Brigham and Women's Hospital, assistant professor of medicine at Harvard Medical School, and a researcher at both the Harvard School of Public Health and the Broad Institute, set out to observe P. falciparum in its natural environment: the human circulation. Using small samples of blood collected from more than 40 malaria patients in Senegal, Daily and her colleagues worked meticulously to devise a method for isolating genetic material from parasites, allowing them to determine which of the nearly 6,000 P. falciparum genes are switched on or off during infection in humans. Importantly, all of the patients involved in the study harbored similar-looking parasites, yet their symptoms varied widely.

These clinical research efforts were led by Professor Souleymane Mboup and Dr. Daouda Ndiaye at Cheikh Anta Diop University. "This

project would not have been possible without the dedicated work of our collaborators in Senegal," said co-author Dyann Wirth, a professor and chairman of the department of immunology and infectious diseases at the Harvard School of Public Health and the co-director of the Broad Institute's Infectious Disease Initiative. "We are grateful to them and to the many malaria patients who generously volunteered to participate in this study."

From the parasites in patients' blood, the researchers simultaneously measured the activity level, or "expression", of every P. falciparum gene. Co-author Elizabeth Winzeler, an associate professor at The Scripps Research Institute, led this aspect of the study. "The ability to look across the parasite's entire genome was essential," said Winzeler. "We uncovered extraordinary things about parasite biology -- things we could not have even imagined."

Winzeler, who is also head of malaria research at the Genomics Institute of the Novartis Research Foundation (GNF), where much of the genomic work was performed, is grateful that organizations like GNF choose to encourage these types of high-risk studies. "We are especially excited about using these observations to guide our drug discovery efforts," she said.

The key to interpreting these results lay in two computational tools, first developed by Mesirov and her colleagues to study the genomics of human cancer cells. By adapting these tools for malaria, the researchers were able to identify distinct groups of parasites, each marked by characteristic sets of active and inactive genes. The biological underpinnings of these groups were made clearer through a second innovative approach: systematically comparing P. falciparum -- whose genes and genome are poorly understood -- to the baker's yeast, an organism that has been extensively characterized at the genetic level. Since the malaria parasite and the baker's yeast are both single-celled

eukaryotes, it is possible they may share some of the same cellular machinery and could also respond in some similar ways to their surroundings.

With this unusual approach, co-senior author Regev and her colleagues were able to describe three different classes of parasites, one of which displayed features associated with a well-known form of parasite metabolism. The other groups, however, were very unusual, reflecting modes of parasite behavior that had never before been described.

One of these novel groups seems to signal parasites that are under extreme environmental stress. Importantly, this group shows a clear correlation with patient symptoms, including high fevers and elevated levels of inflammatory markers in the blood. "This is a remarkable result -- it suggests the malaria parasite can sense what is happening within its host and adjust its biology accordingly," said Daily. "That interaction signals a fundamental shift in the way we think about malaria, one which will hopefully lead to more effective treatments -- particularly for the most severe cases of the disease."

The other parasite group is associated with an alternative form of parasite metabolism, which relies on two specialized cellular compartments called the mitochondria and the apicoplast. That result is particularly surprising since mitochondria in P. falciparum were previously thought to be non-functional.

"For decades, our knowledge of the parasite has been driven solely by studies in cultured cells, not in humans," said Wirth. "Our work underscores the importance of studying the malaria parasite in its natural environment and will hopefully spark novel approaches to malaria drug discovery."

Source: Broad Institute of MIT and Harvard