Finding malaria's weak spot

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A ground-breaking imaging system to track malarial infection of blood cells in real time has been created by a collaboration catalysed by the University's Physics of Medicine Initiative.After over a decade of research into malaria, biologists Dr Teresa Tiffert and Dr Virgilio Lew at the Department of Physiology, Development and Neuroscience found their efforts to observe a key stage of the infection cycle severely hindered by the limits of available technology. An innovative collaboration with physicist Dr Pietro Cicuta at the Cavendish Laboratory and bio-imaging specialist Professor Clemens Kaminski in the Department of Chemical Engineering and Biotechnology is now yielding new insights into this devastating disease.

Under attack

Malaria is caused by parasites transmitted to humans through the bites of infected mosquitoes. According to the *World Malaria Report 2011*, there were about 216 million cases of malaria causing an estimated 655,000 deaths in 2010. Tiffert and Lew established their malaria laboratory in Cambridge in 1999 to investigate the most deadly form of the parasite, Plasmodium falciparum. Becoming increasingly resistant to available drugs, this species in particular is a growing public health concern.

Their current focus is a mysterious step in the life cycle of P. falciparum occurring inside the infected human's bloodstream. The parasites, at this stage called merozoites, attach to and enter red blood cells (RBCs) to develop and multiply. After two days, the new merozoites are released and infect neighbouring RBCs. Over several days, this process amplifies

the number of parasitised RBCs and causes severe and potentially lethal symptoms in humans.

"A huge amount of research has been devoted to understanding the RBC penetration process," said Tiffert. "The focus of many vaccine efforts is the molecules on the surfaces of both parasite and red cell that are instrumental in recognition and penetration. Our collaboration with Clemens developed new imaging approaches to investigate what happens in the cells after invasion. But the pre-invasion stage, when a merozoite first contacts a cell targeted for invasion, remained a profound mystery. Our research indicates that this stage is absolutely critical in determining the proportion of cells that will be infected in an individual."

For invasion to occur, the tip of the merozoite has to be aligned perpendicularly to the RBC membrane. Tiffert and Lew are focusing on how this alignment comes about, which has proved a formidable technical challenge. "The merozoites are only in the bloodstream for less than two minutes, where they are vulnerable to attack by the host's immune system, before entering a RBC. To investigate what is going on we need to record lots of pre-invasion and penetration sequences at high speed, using high magnification and variable focusing in three dimensions. And the real challenge is to have the microscope on the right settings and to be recording at exactly the time when an infected cell has burst and released merozoites – something that is impossible to predict," said Tiffert.

Techniques used by previous investigators have produced few useful recordings of this process occurring in culture, but from these an astonishing picture is emerging. "The contact of the merozoite with the RBC elicits vigorous shape changes in the cell, not seen in any other context," said Lew. "It seems clear that this helps the merozoite orientate itself correctly for penetration, because all movement stops as soon as this happens. The parasite is somehow getting the RBC to help it

invade."

A collaborative approach

Cicuta, a University Lecturer involved in the University's Physics of Medicine Initiative – which is bringing together researchers working at the interface of physical sciences, life sciences and clinical sciences – met the trio by chance three years ago. He realised he could use his background in fundamental physics to pioneer a new approach to understanding malaria. "It's been a gradual move for me to apply what I've learnt in physics to biology," he said. "From the physics point of view, RBC membranes are a material. This material is very soft and undergoes deformations and fluctuations, and I was interested in understanding the mechanics involved during infection with malaria."

Drawing on his expertise in the development of experimental techniques, Cicuta collaborated with Tiffert, Lew and Kaminski to pioneer a completely automated imaging system that pushes the boundaries of live cell imaging, enabling individual RBCs and merozoites to be observed throughout the process of infection. The research was funded by the Biotechnology and Biological Sciences Research Council and the Engineering and Physical Sciences Research Council.

"This microscope can not only run by itself for days, it can perform all the tasks that a human would otherwise be doing. It can refocus, it can find infected cells and zoom in, and when it detects a release of parasites it can change its imaging modality by going into a high frame-rate acquisition. And when the release has finished it can search around in the culture to find another cell to monitor automatically," said Cicuta. "We also want to integrate a technique called an optical trap, which uses a laser beam to grab cells and move them around, so we can deliver the parasites to the cells ourselves and see how they invade."

"So far, we've been able to gather over 50 videos of infections, which my PhD student Alex Crick has processed to show very clearly that the RBCs undergo large changes in shape when the merozoites touch them. We've also seen very strange shape changes just before the parasites come out of the cells, and we want to see whether this has a bearing on the parasites' ability to infect subsequent cells."

During the development of the microscope, the team discovered variability in the way the infected RBCs behave before they burst. "It's important to know that there isn't just one story. The only way to find this out is to look at many cells, which this system allows," said Lew. "It's a new level of data that allows us to get experimentally significant results, and better understand the diversity of the merozoites," Cicuta added.

Used in conjunction with other tools such as fluorescent indicators and molecular biological tools, the new technology will allow Tiffert and Lew to test their hypotheses about the pre-invasion stage of the disease. They hope to determine the critical steps, which could provide clues as to how to stop an infection. "This microscope is an extraordinary new tool that has potential for use across a huge field of biological problems involving cellular interactions," explained Lew.

"It may provide a route to designing effective antimalarial drugs, reducing invasive efficiency and decreasing mortality," said Tiffert. "The automation we have achieved with this microscope will also be very important for future testing of malaria drugs and vaccines," added Cicuta.

A visionary initiative

"The Physics of Medicine Initiative has been essential to our work," said Cicuta. The University formally established the Initiative in December

2008 through the opening of a new purpose-built research facility adjacent to the Cavendish Laboratory, funded by the University and The Wolfson Foundation. The goal is to break down traditional barriers that have tended to limit interactions between researchers in the physical and biomedical sciences.

"I met my collaborators through a Physics of Medicine symposium, and the new building is the only place in the University where this type of research can be done," added Cicuta. "It's set up for safe handling of hazardous biological organisms like P. falciparum, and also has the facilities to design hardware for our advanced microscopes. This work is exciting because it's interdisciplinary. By applying physics to the knowledge biologists have been developing for many years, we can make very fast progress."

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