

Challenging times for malaria vaccines

Malaria is a major killer and causes immense suffering throughout the world. As **Sarah Gilbert** describes, without willing volunteers, it would be impossible to trial the exciting new vaccines to prevent this disease which are currently under development.



More than 2 billion people worldwide are at risk of infection with *Plasmodium falciparum* malaria and 500 million clinical attacks occur each year. In Africa malaria causes the deaths of children as well as leaving some of the survivors with long-term neurological impairment. It causes children to miss school and parents to miss work while they care for sick children. It is a disease of poverty and a cause of poverty. Large numbers of people do not have access to effective drugs and cannot afford to pay for them. An effective vaccine given early in childhood along with other childhood immunizations would have a major effect on health care programmes for areas affected by *P. falciparum* malaria, but as yet, no such vaccine exists.

There are many difficulties to be overcome in developing a malaria vaccine. The parasite has a complex life cycle, part of which occurs in mosquitoes, and has evolved to

both suppress and evade the human immune system. Many attempts to make a vaccine have failed. However, scientists developing vaccines to act against the liver stage of malaria have a tool available to them that allows the protective efficacy of new vaccines to be tested at an early stage in a short period of time: human challenge with infectious malaria parasites.

Which stage of the life cycle to attack?

When a mosquito carrying malaria parasites (the stage known as the sporozoite) in its salivary glands bites someone, small numbers of sporozoites enter the body and quickly move through the bloodstream to the liver, where they invade the cells. Over the course of a week, during which the infected person has no symptoms, the parasites multiply inside the liver cells before breaking out into the blood stream in large numbers to begin the blood-stage infection. Red blood cells become infected, then parasites multiply further

▲ Macro photograph of an *Anopheles stephensi* mosquito feeding on human skin. Sinclair Stammers / Science Photo Library

inside them and break out again, causing waves of fever, and eventually anaemia or blockage of the micro-circulation by parasitized red cells.

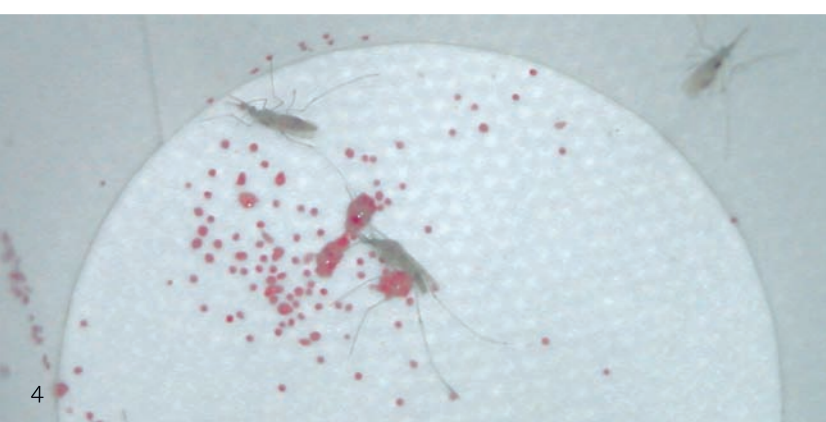
Some malaria vaccines are designed to attack either the sporozoite stage as it enters the body, or the liver stage. Vaccines that are 100% effective at either of these stages will clear the infection before symptoms arise, and no parasites will ever emerge to initiate blood-stage infection. Slightly less effective vaccines will result in a reduction of parasites at the start of

the blood-stage infection, which then take longer to become detectable in the blood than if the vaccine had had no effect. This is a useful result, as it shows that the vaccine is working to a large extent, and is a candidate for further improvement. It is therefore possible to deliberately infect volunteers who have been immunized with new malaria vaccines to test the efficacy of the vaccines. After 7 days, blood samples are taken twice daily to screen for the presence of parasites. If any are detected, the vaccine has failed, and the volunteer is immediately treated with an effective anti-malarial. If none are detected by day 21 following infection, the vaccine

has been completely effective in that volunteer.

Oxford malaria challenge trials

Carrying out these trials is not a simple matter, but more than 10 such trials have now taken place at the Centre for Clinical Vaccinology and Tropical Medicine at Oxford University. Here, a research team led by Professor Adrian Hill has been developing vaccines to induce protective T-cell responses that can kill infected liver cells and the parasites within them. Following extensive pre-clinical studies, a number of vaccines expressing malaria liver-stage antigens from either DNA



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vaccines or recombinant, replication-deficient viral vectors have been tested in volunteers. Each new vaccine for testing must be manufactured under suitable conditions and undergo extensive quality control and toxicology testing before permission to test it in healthy volunteers is obtained. A new vaccine is first tested in a small number of volunteers who are carefully monitored for any side effects before immunizing a larger number of people. Blood samples are taken before and after vaccination to establish the effect of the vaccine on the T-cell response to the antigen in the vaccine. If the results are good, a challenge trial follows.

In malaria-endemic areas, malaria follows a bite from an infected mosquito, and that is the way the volunteers are infected as well, although to ensure that everyone gets a high enough inoculum to become infected, five mosquitoes per person are used. The challenge takes place in the insectary of Imperial College, London, under the supervision of Professor Bob Sinden. For the volunteers, that means a day trip to London with an early start from Oxford railway station. To make things more manageable the challenge takes place over 2 days, with up to 15 volunteers taking part each day. Some of these will have been immunized with the vaccine regime under test, whereas others are acting as 'infection controls' to check that people with no immunity to malaria all get infected after the challenge – and so far, they always have. Waiting for the volunteers to arrive at the railway station is always a nerve-wracking time for the research team. Occasionally people get cold feet at the last moment and decide not to go ahead with the experiment. Replacement 'infection controls' may then be called in at the last minute from a reserve list, but if immunized volunteers drop out, they cannot be replaced.

Feeding time for the mosquitoes

Once everyone arrives at Imperial College, volunteers take it in turns to rest their arm (unwashed, to encourage mosquito biting) over a paper coffee cup containing mosquitoes under an open mesh lid, which keeps them inside, but allows them to bite. They prefer darkness, so a cloth is draped over the arm for a few minutes, and then the mosquitoes are examined to see how many have fed on the volunteer. Some people are more attractive to the mosquitoes than others. The mosquitoes have not been fed before the challenge, so

they should be hungry, but if they don't bite at first, rubbing a sweaty sock over the arm usually attracts them!

After feeding, the mosquitoes are dissected to ensure that they were suitably well infected, and if not, the volunteer is invited back for more bites until everyone has received bites of five well infected mosquitoes. After that, it's back on the train to Oxford and normal life for the next 6 days while the parasites incubate. Then there's a week of frantic activity for the research team, seeing the volunteers twice daily, taking blood samples, preparing and reading blood films and carrying out highly sensitive real-time PCR assays to give a quantitative measure of blood-stage parasitaemia during the very early days of blood-stage infection. By day 14, all the controls have been diagnosed as infected and given treatment, and everyone is waiting to see how many immunized volunteers will make it to day 21 without a parasite being found in their blood. No one making a diagnosis knows who was immunized or with which regime, but as the numbers gradually decrease everyone is placing their bets as to what worked and what didn't. On day 21, the remaining volunteers are declared protected from malaria, and the vaccine is deemed to have worked, but the volunteers then receive drug treatment for malaria as a safeguard.

Following up

Every trial is followed by a dinner for the volunteers and research team, to

thank the volunteers, compare notes and receive feedback. *P. falciparum* malaria does not recur, so once the volunteers are treated they are free of the parasite for good. That's the end of their involvement in the process, but for the research team it is just the start of a great deal of analysis, and plans for the next trial, whether that means taking the vaccine into trials in Africa or going back to the drawing board. Gradually, progress is being made, and we are learning how to make more effective vaccines.

T-cells can also protect against other diseases, such as HIV, tuberculosis and cancer, where there are no human challenge models available. The outcome of this work may therefore be not only a vaccine against malaria, but a series of new prophylactic and therapeutic vaccines that build on the results of these challenge trials.

Dr Sarah Gilbert

Hill Group, The Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford, OX3 7BN, UK (e sarah.gilbert@well.ox.ac.uk)

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Further reading

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◀ A series of photographs illustrating the volunteer infection process. 1. Hungry, infected mosquitoes wait under a mesh draped over a paper coffee cup. 2. A volunteer rests his arm over the cup to allow the mosquitoes to feed. 3. The effect of a successful feed on the volunteer's arm. 4. The mosquitoes in the paper cup after their blood meal. Dan Webster