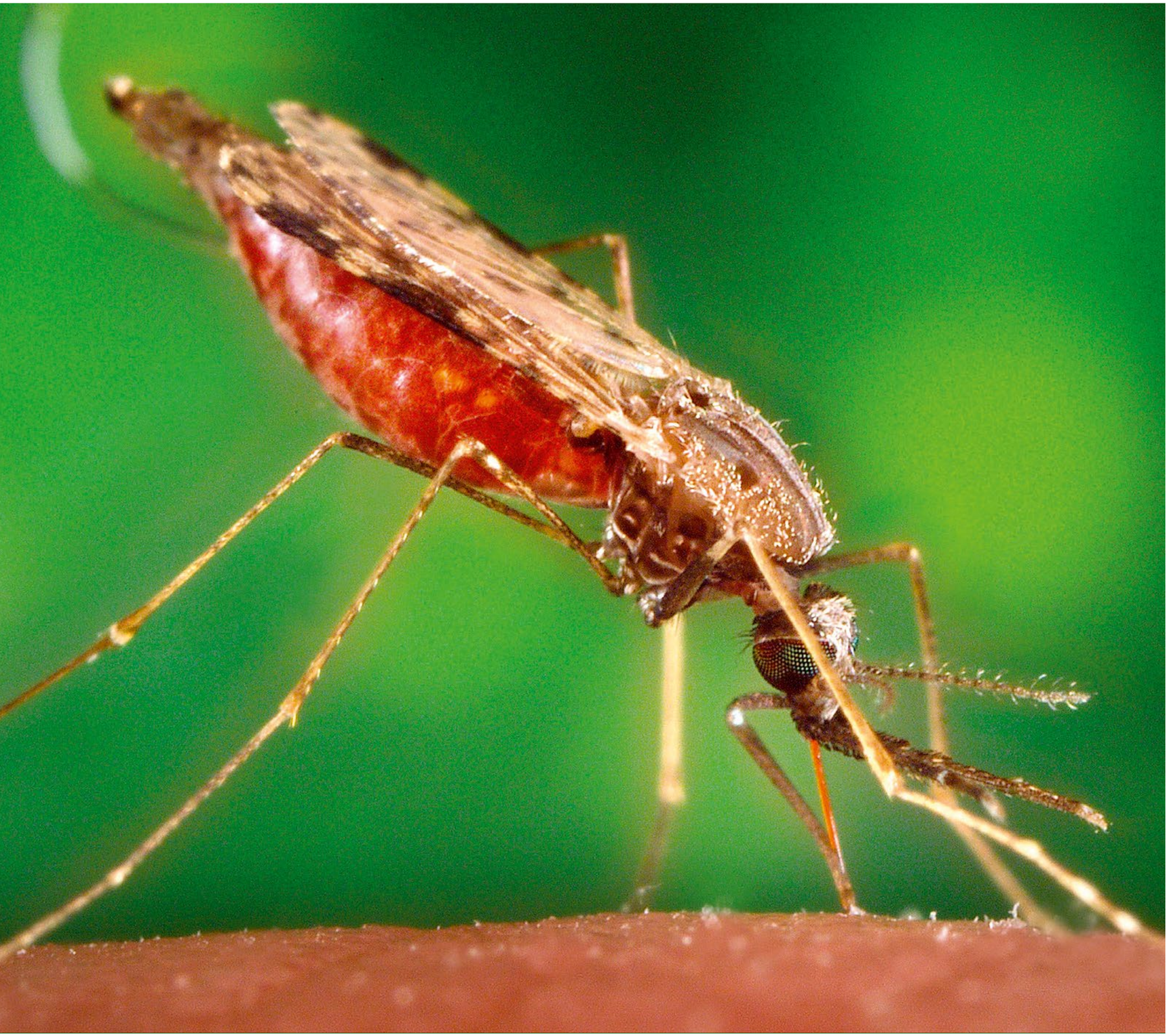


O so simple...
How blood group O fights off malaria

Professor Mats Wahlgren's recent work on the mechanisms and proteins involved in the development of severe malaria has advanced current scientific understanding of the disease and may reveal answers that will allow a vaccine to be developed.

Malaria is a widely geographically distributed disease – though most common in sub-Saharan Africa – that is estimated by the World Health Organization to affect 200 million people every year, 425,000 fatally. The majority of those who die from malaria are under five years old, making malaria one of the most important diseases to eliminate in order to improve childhood mortality.

The disease is caused by vector-borne parasites in the *Plasmodium* family, and most cases of fatal or severe malaria are caused by *Plasmodium falciparum*. Severe cerebral malaria is characterised by the occlusion of cerebral vessels by tightly packed red blood cells. Blood clots block vessels, causing coma, brain damage and ultimately death.

REVEALING THE MECHANISMS

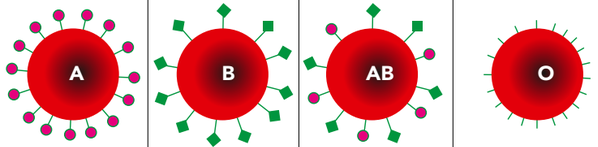
Blood groups are classified according to the way different antigenic substances are expressed on the surface of red blood cells, the vascular endothelium and serum proteins. This means that red blood cells of different blood groups react differently to exposure to different proteins because differently shaped complexes are exposed on the cell surfaces. It was demonstrated in the late eighties and early nineties that those with O type blood are protected from severe or fatal malaria. However, the exact processes that facilitate this protection had until relatively recently eluded scientists. In research published last year, a team from the Karolinska Institutet in Sweden led by Professor Mats Wahlgren, have pushed current understanding of the underlying mechanisms of malarial pathogenesis one step further.

Some of their work during the early nineties highlighted the importance of 'rosetting' as a phenomenon related to malaria. Rosetting is a process whereby uninfected red blood

RIFINs may be prime candidates for targeting in the search for a drug for the treatment of severe malaria


The Mats Wahlgren research group

The team from the Karolinska Institutet have pushed current understanding of the underlying mechanisms of malarial pathogenesis one step further

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in Plasma	Anti-B	Anti-A	None	Anti-A and Anti-B
Antigens in Red Blood Cell	A antigen	B antigen	A and B antigens	None

cells adhere to red blood cells that have been infected with malarial parasites such as *P. falciparum*, forming clusters, or rosettes. Once rosettes are formed, it becomes more difficult for the host's immune system to destroy the infected cells because they are surrounded by uninfected cells.

In a paper published in *The Lancet* in 1990, Johan Carlson, Mats Wahlgren and colleagues showed that isolates from children with severe malaria displayed higher rosetting activity compared with isolates from children with milder forms of the disease. Findings such as these emphasised the importance of further research into the mechanisms behind rosetting and the prevalence of anti-rosetting antibodies. Later work revealed that rosetting was less pronounced in blood group O than in blood groups B, and particularly A.

MAKING PROGRESS

Published in *Nature Medicine* in 2015, Wahlgren's team's new research further develops the understanding of the disease. In what Wahlgren describes as a 'conceptually simple' mechanism, they found that polypeptide proteins called RIFINs (repetitive interspersed family of proteins) secreted by parasites move to the surface of infected blood cells, causing them to become sticky and bind together, creating the clots that block blood vessels and cause the symptoms of severe malaria. Interestingly, these proteins bind more strongly to blood cells belonging to blood group A than to those in group O.

Rosettes can be composed of more than ten blood cells, and are much larger in infected individuals with blood type A than in those with type O blood.

Rosetting was previously attributed mainly to the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), although the recent study suggests that RIFINs are equally, if not more, important in causing blood cells to adhere to each other. This is particularly the case in blood group A: the majority (70%) of RIFINs belong to a subgroup, A-RIFIN, which preferentially cause red blood cells with A antigens to adhere to each other, creating large rosettes. While this mechanism is also seen in O blood group red blood cells, the rosettes generated are much smaller.

The function of RIFINs had not previously been identified, yet they are encoded by 150 rif genes and comprise the largest family of antigenically variable molecules in the *P. falciparum* parasite that causes severe malaria. The team's analysis indicates that the incidences of RIFIN and PfEMP1 at the surface of infected red blood cells correlated with one another as well as with rosetting rates, suggesting that both are implicated in the formation of rosettes.

However, they show that A-RIFINs are the major ligand, or binding molecule, of the process in blood group A. This is demonstrated by the continued formation of rosettes in blood group A once PfEMP1 was removed by treatment with the enzyme trypsin or blocked by the addition of PfEMP1-specific antibodies. Individuals with blood type O are able to generate antibodies to counteract PfEMP1, which disrupts the rosettes and prevents these individuals developing severe malaria. All of this means, simply, that A-RIFINs mediate rosetting in group A, whereas PfEMP1 is mainly implicated in group O rosetting, though rosetting is much more minimal in group O.

LOOKING AHEAD

The team's findings fit well in the current body of literature and have been well received. They support the hypothesis that O-type alleles have been evolutionarily selected in areas with high incidences of malarial infection and suggest that RIFINs may contribute to the varying global distribution of ABO blood groups by selecting preferentially for group O.

As Wahlgren says, 'we can explain the mechanism behind the protection that blood group O provides against severe malaria, which can, in turn, explain why the blood type is so common in the areas where malaria is common. In Nigeria, for instance, more than half of the population belongs to the blood group O, which protects against malaria'.

Furthermore, the team's work suggests that RIFINs may be prime candidates for targeting during screening for new drugs and may be the key to unlocking doors in the search for a vaccine for malaria.

Q&A

How does your research fit into the current drive against malaria?
Forty percent of the world's population is at risk of malaria, and 214 million acquire the infection annually. An additional 400 million are asymptomatic carriers of malaria parasites. Each year, four million cases of severe malaria occur, of which 10-20% (430,000) succumb to the infection, representing a daily death toll of 1000 to 1500 that is predominantly made up of children. Without treatment, cerebral malaria is invariably fatal. However, there is a genuine lack of understanding of how severe malaria is brought about, what triggers the pathogenesis of severe malaria? We therefore both need a detailed knowledge of the pathogenic processes, and adjunct drugs that would decrease the number of deaths and sequel from severe malaria. We are working in these areas and I was one of the founders of Dilaforette Ltd, a company which develops adjunct therapy for severe malaria and sickle-cell disease.

Your project has made some significant steps in explaining how blood group O offers protection against malaria. Are you proud of your team's achievement?
We are proud of our work and also happy that a recent study in *Nature Medicine* also shows that blood group O is selected for in populations where malaria is highly endemic. That strengthens our data and indeed suggests that both RIFINs (repetitive interspersed family of proteins) and ABO blood groups are at the centre-stage of the pathogenesis of severe malaria.

What were the key challenges that you managed to overcome?
Identifying the RIFINs as the adhesive molecule that binds to the ABO blood-group antigens was difficult since there are about 150 different RIFINs and only one of these is relevant, the one expressed. 149 genes are silent.

The malaria parasite can make red blood cells sticky. Your team identified an important mechanism for how this occurs – how might this be applied to other research?
The biochemistry of the molecule is interesting and the role of the molecule in adhesion also argues that other molecules such as the STEVORs are important for the survival of the parasite but also points towards its involvement in the pathogenesis of malaria.

What are the next steps towards applying your work to fight the disease?
It would be of importance to develop a vaccine from the RIFINs that could protect against severe malaria. However, we will only know after further work if that is doable.

Detail

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BIO
Mats Wahlgren is a professor of infectious disease control and parasitology at the Karolinska Institutet in Stockholm, where he leads a research group. Their research focuses on the molecular pathogenesis of severe malaria caused by *Plasmodium falciparum*, looking particularly at the effect of the disease on the surfaces of infected red blood cells. The team was responsible for linking the rosetting phenomenon with severe malaria, and is currently working with a pharmaceutical company, Dilaforette AB, to develop an anti-rosetting vaccine. They also collaborate closely with scientists from Makerere University Medical Biotech Labs in Kampala, Uganda, as well as others from numerous other countries.

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