The mechanisms of malaria

*Plasmodium falciparum* malaria kills around 1 million people every year, but the reasons why some individuals die from the infection while others do not even develop symptoms are poorly understood. Dr Fousseyni Touré Ndouo is investigating the immunological mechanisms behind this puzzling phenomenon.

Can you outline the overarching aims and objectives of your research? How has your focus evolved over the course of your career?

Our research aims to investigate the molecular mechanisms involved in severe malaria in order to identify parasite virulent encoded molecules which could be targeted for antimalarial drug and/or vaccine development.

What is currently understood about why some people that become infected with malaria remain asymptomatic whereas others develop severe malaria and die?

Little is known about that. However, the patient’s clinical outcome is strongly associated with many factors, including those related to the parasite, such as invasion pathways, cytoadherence (cell-to-cell contact) and antigenic variation; those related to the host, including immunity, genetic factors, age and pregnancy; and environmental factors such as transmission intensity, access to treatment, economy and cultural factors. The adhesion of *P. falciparum* parasitised red blood cells (PRBC) to the host’s endothelial cells induces three outcomes: the mechanical blockade of micro vessels; the over-production of inflammatory cytokines, and host endothelial cell activation and apoptosis. Moreover, the malarial pigment haemozoin induces apoptosis of the red blood cell precursor.

In what ways are you making use of this differential symptom burden to better understand the pathology of severe malaria?

We study the pathogenicity of *P. falciparum* field isolates freshly collected from infected individuals who are both asymptomatic and symptomatic. Our studies involve analysis of the consequences of cytoadherence-induced signalling and the identification of genes involved in severe malaria pathogenesis.

To what extent has your research elucidated the molecular mechanisms by which PRBCs adhere to vascular endothelial cells resulting in enhanced clinical severity?

Using host endothelial cell and PRBC co-cultures, we have previously shown that host endothelial cells triggered by cytoadherence may lead to neurological complications in *P. falciparum* malaria. However, only 20 per cent of *P. falciparum* isolates from symptomatic individuals induced host endothelial cell apoptosis. These previous results suggest that uncomplicated or asymptomatic patients harbouring apopogenic PRBCs may progress more rapidly to severe malaria than those carrying non apopogenic PRBCs. We have also shown that cytoadherence is necessary but not sufficient to explain all the damage caused to the host endothelial cells. In other words, *P. falciparum* can induce host endothelial cell apoptosis without cytoadherence.

Can you explain the causes and consequences of endothelial cell apoptosis? Are there particular parts of the body that are more severely affected than others?

Host endothelial cell apoptosis is caused by the activation of the cell death machinery when parasite ligands bind to the specific receptor on the endothelial cell’s surface. This process could be associated with blood-brain barrier impairment. In *P. falciparum* malaria it has been demonstrated that apoptosis occurs more frequently in brain and lung endothelial cells in addition to the red blood cell precursor.

What led you to investigate a potential epidemiological interaction between hepatitis B virus, hepatitis C virus and *P. falciparum*?

We were led to investigate this interaction because of a prior awareness that the molecular interaction between these pathogens, which share the same ecological niche, would facilitate the development of novel strategies to combat them.

How does the immune system react to the simultaneous presence of these pathogens in the liver and in what way does this affect the development of the malaria parasite?

Two opposing hypotheses have been proposed. The first is that the immune system reduces the human leukocyte antigen within infected hepatocytes, therefore protecting the malaria parasite from destruction. In contrast, the second is that the malaria parasite is inhibited by the induction of non-specific inflammatory factors. Our results show that hepatitis C virus infection may lead to the slower emergence of *P. falciparum* into the patient’s blood. This ‘protection’ may be due to the reduction of sporozoites entering hepatocytes and/or the overexpression of interleukins, such as interferon gamma, in hepatitis C positive individuals.

What are your research objectives for the near future?

We are designing clinical trials of rho kinase inhibitors for the management of severe malaria. Our immunogenicity studies of parasite ligand peptides and investigations of *P. falciparum* pathogenicity (using at least three types of human endothelial cells) are underway. Understanding the molecular mechanisms behind the interaction between host and parasite could provide new insights for malaria treatment strategies.
In the ongoing fight against malaria in West Africa, researchers at the Centre International de Recherches Médicales de Franceville, Gabon, are investigating the therapeutic potential of Fasudil, which can prevent the endothelial cell apoptosis caused by severe *Plasmodium falciparum* infection.

**INFECTION BY THE** malaria parasite, *Plasmodium falciparum*, can lead to life-threatening forms of this disease, such as cerebral malaria – caused by the agglutination of parasitised red blood cells (PRBC) in the microvasculature of organs such as the brain and lungs, and their adherence to the endothelial cells lining the blood vessels in these organs. The adhesion of PRBCs to the host's endothelial cells directly activates the rho kinase signalling pathway and induces the production of reactive oxygen species, leading to endothelial cell death and the subsequent detrimental disruption of the blood-brain barrier.

Despite the current efficiency of antimalarial drugs, such as quinine and artemisinin derivatives, in destroying parasites, *P. falciparum* malaria still causes up to 1 million deaths every year. Even when parasites have been eradicated from the host’s system, the condition remains fatal for up to 20 per cent of patients, and others suffer from persistent associated symptoms. Little is known about why some non-immune individuals die whereas others have uncomplicated or even asymptomatic infections; further research is needed to elucidate the mechanisms underlying this. In addition, a new strategy targeting both parasite elimination and endothelial cell protection is urgently needed in the field.

**STUDYING THE EFFECTS OF FASUDIL**

In his current project, Touré Ndouo is investigating the antimalarial therapeutic potential of Fasudil, a rho kinase inhibitor already in clinical use for the treatment of cardio- and neurovascular diseases. Fasudil has been successfully tested on laboratory strains of *P. falciparum*, *in vitro*, to demonstrate its ability to protect the integrity of endothelial cells and to reverse endothelium damage caused by the activity of PALPFs. Touré Ndouo’s team has been assessing whether this drug would have a similar effect on *P. falciparum* isolates taken directly from malaria patients.

The researchers recruited 300 symptomatic children aged from one to 14 years, 54 of whom were found to be infected with *P. falciparum*. Of these, 53 were cases of uncomplicated malaria and one case of severe malaria. The team used two approaches to examine the effect of Fasudil on endothelial cell apoptosis caused by red blood cell *P. falciparum* infection in the participating patients. Contact co-culture of HLECs and PRBCs was used to measure apoptosis mediated by cytoadherence, and non-contact experiments were carried out to examine the role of an alternative apoptotic mechanism – that which works through the action of diffusible stimuli.

**MECHANISMS OF MALARIA**

At the Centre International de Recherches Médicales de Franceville in Gabon, West Africa, one research group is studying the pathophysiological mechanisms of severe malaria in the lung and brain, with a particular focus on the interaction between parasite ligands and host cell receptors; knowledge of which is crucial for designing specific pathway inhibitors that could prevent the development of this fatal disease. Led by Dr Fousseyni Touré Ndouo, the group uses human lung endothelial cells (HLECs) to study the pathophysiology of *P. falciparum* malaria *in vitro*. 
FIELD ISOLATE RESULTS

Touré Ndouo’s team has been able to show, for the first time, that soluble factors released by *P. falciparum*-infected red blood cells can trigger HLEC apoptosis and, as such, cytoadherence is not a necessity for the development of severe malaria. They found that 23 per cent of the field isolates collected from different individuals exhibited HLEC apoptosis; 57 per cent of which acted via cytoadherence and 43 per cent via parasite-derived soluble factors. Furthermore, this apoptosis was reduced in both contact and non-contact conditions by an average of 68 per cent. Cytoadherence-mediated HLEC apoptosis was rapidly detectable in patient samples after only four hours of co-culture with most apoptogenic *P. falciparum* strains. Contrastingly, for a small minority of strains it took over 24 hours for apoptosis detection. As the same number of PRBCs were used in the experiments for each isolate, this difference in the timing of apoptosis onset is likely due to the nature and expression kinetics of the PALPF involved: “We postulate that rapid transducer ligands and slow transducer ligands may exist. If so, the nature and affinities of HLEC receptors, recognised by the two categories of ligand, are also likely to be different,” Touré Ndouo adds. Comparing their work using isolates from field studies and laboratory strains of *P. falciparum* Touré Ndouo explains: “We believe that field isolates exhibit different behaviour to previously investigated laboratory *P. falciparum* strains. These innate behavioural characteristics remain intrinsic to the parasites and are influenced by environmental factors such as vector, host or ecology”.

To further probe the contrasting induction of apoptosis, the group carried out transcriptome analysis of those PRBCs which induce HLEC apoptosis through cytoadherence. Considering the cause of HLEC apoptosis induction, the results revealed that *P. falciparum* field isolates may have a greater propensity for cytoadherence or soluble factor release, but do not make use of both mechanisms.

TOWARDS MALARIA THERAPEUTICS

These results show that the interaction between HLECs and *P. falciparum* is more complicated than previously thought. However, one of the most important findings of Touré Ndouo’s study is that Fasudil prevented HLEC apoptosis triggered by both cell-cell contact and soluble factors, without preventing PRBC cytoadherence. This is mediated by Fasudil inhibition of rho kinase phosphorylation of enzymes involved in HLEC activation. “This inhibition of the rho kinase signalling pathway may be sufficient to inhibit PRBC-mediated HLEC apoptosis induced both by cytoadherence and by diffusible factors, assuming that rho kinase is activated by both stimuli,” he explains. Furthermore, rho kinase inhibition by Fasudil was found to inhibit nitric oxide production. A low nitric oxide environment can exert an antiapoptogenic effect on several cell types, including endothelia, and could therefore be a potential therapeutic route to explore in future studies.

TO INHIBITION OF *PLASMODIUM FALCIPARUM* FIELD ISOLATES-MEDIATED ENDOTHELIAL CELL APOPTOSIS BY FASUDIL: THERAPEUTIC IMPLICATIONS FOR SEVERE MALARIA

OBJECTIVES

To investigate the molecular mechanisms involved in severe malaria and characterise the parasite ligands associated with endothelial activation and/or the death signalling pathway. To elucidate the molecular mechanisms involved in these endothelial dysfunctions to enable the identification of new target molecules for chemotherapeutic drug design, or antigens for vaccine candidates.

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