ISGlobal
Barcelona Institute for Global Health

The Pillars of the Earth: How Basic Science Contributes to the Fight Against Malaria

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Looking for the Unexpected

Basic scientific research is done to advance our knowledge and understanding of a particular topic or problem, but it does not necessarily produce results that have any immediate practical application in health care. However, the knowledge acquired often lays the groundwork for advances that help us to move in the right direction, opening the door to new discoveries that are directly applicable to patient care or disease prevention.

Basic science is essential because it is an integral part of a continuum that cannot be broken down without rendering it useless. Despite this, in medicine it is customary to divide research into separate phases, each with its own name: thus we have basic, translational, preclinical and clinical science. In practice, however, all these phases form an inseparable whole that can only work on the basis of constant feedback.

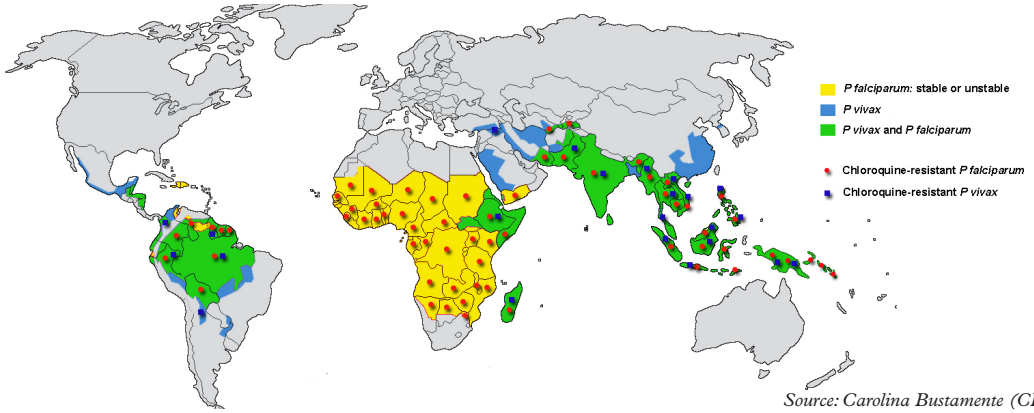
So why do so many people find it hard to recognise the great social value of basic scientific research? There are many reasons, but two stand out. Firstly, the force that drives basic research is a delicate balance between pure curiosity and a desire to be useful. While curiosity has provided humanity with amazing discoveries, most agencies now place a low priority on research that does not promise any immediate practical result.

The Impact of Malaria

Malaria is caused by protozoan parasites of the genus *Plasmodium*. Five species of *Plasmodium* cause malaria in humans: *P falciparum*, *P vivax*, *P malariae*, *P ovale* and *P knowlesi*, but P *falciparum* and *P vivax* are responsible for most of the burden of disease in the world (Figure 1). *P falciparum* is the cause of more than 200 million clinical cases of malaria and one million deaths a year, especially in children under five in sub-Saharan Africa, and it can cause serious complications in foetal development in pregnant women and even maternal deaths. *P vivax*, the most common malaria parasite, is widely distributed throughout the world and is responsible for about 100 to 300 million clinical cases every year, including cases of severe illness and death.

The fight against malaria has grown increasingly difficult as the parasites develop resistance to antimalarial drugs such as chloroquine (Figure 1) and because of complex interactions with other infections prevalent in areas where malaria is endemic, including human immunodeficiency virus (HIV).

Figure 1: Malaria and chloroquine-resistant disease: worldwide distribution



Source: Carolina Bustamente (CRESIB)

Second, basic science is the discipline in which the connection between the results and their practical application is the least obvious or foreseeable: the issue is not that there is no link, but that an explanation is required to make the connection apparent.

The malaria parasite resists ...

Despite advances in recent years, malaria continues to resist control and elimination. We are still searching for a highly effective vaccine that is easy to administer, inexpensive and widely accessible to those most in need: vulnerable people living in situations where they are at risk of infection. While we do have effective drugs to treat people with the disease, the pathogen that causes malaria is capable of developing resistance to these treatments.

In the same way that antibiotics, such as penicillin, have gradually become ineffective against many bacteria, the protozoan parasites that cause malaria develop resistance to all the drugs currently used to eliminate them. The race to update the arsenal of antimalarial drugs is complicated by the perverse physiology of the parasite and geo-economics conditions. Besides spending most of its life safely hidden inside red blood cells, *Plasmodium* parasites spend part of their life cycle inside mosquitoes of the genus *Anopheles;* to add insult to injury, the great apes are infected by *Plasmodium* species that, in certain circumstances, can also infect humans. Finally, even if all the effort invested in developing a new weapon came to fruition, it might still not be enough to solve the problem because any new drug would have to be not only effective but also inexpensive. As virtually all cases of malaria occur in impoverished regions of the world, the production of antimalarials is not an attractive proposition for large pharmaceutical companies.

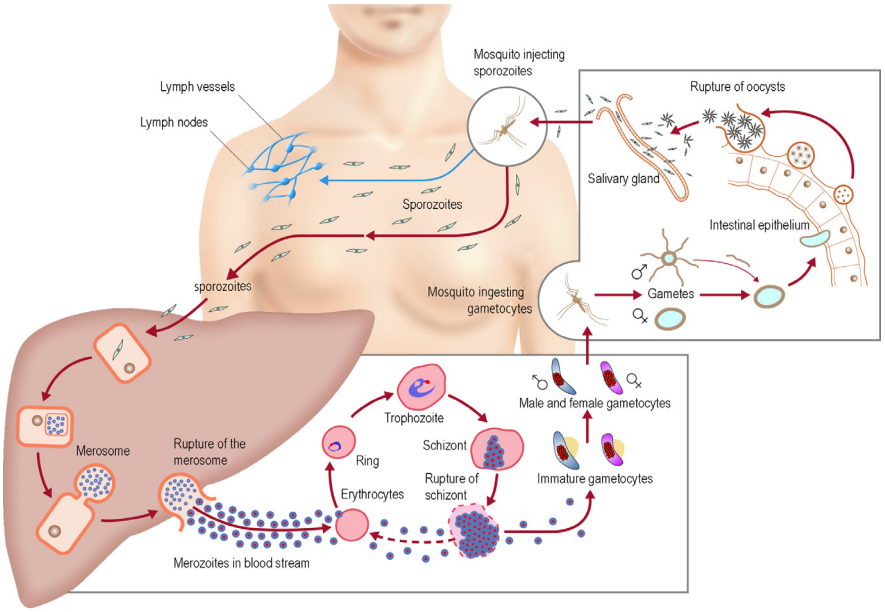
The malaria microbe has developed sophisticated mechanisms to avoid being eliminated by the immune system. The human body develops natural immunity to many diseases after only a single infection, a mechanism that provides the basis for effective vaccines. In the case of malaria, however, a single infection does not make us resistant.

Repeated infection is required to generate immunity to malaria: children who live in areas where the disease is common and endemic gradually develop resistance to different forms of the disease, although protection is never complete. Therefore, to develop an effective vaccine it is not enough to induce the immune responses that occur after a single infection, which would be sufficient in the case of certain other diseases.

The First Malaria Vaccine

RTS,S is the most clinically advanced malaria vaccine candidate. Developed by GlaxoSmithKline in collaboration with PATH-MVI and several international research institutions (including CRESIB), the RTS,S vaccine candidate has been shown to be safe and effective, with an efficacy against clinical malaria of around 50% in numerous phase I and II clinical trials. The vaccine is currently being tested in Africa in a large multicentre phase III trial, the last stage in clinical development before submission to the regulatory authorities. In spite of promising results, the effectiveness of the vaccine is still partial, the duration of protection is limited, and the mechanism of action is still poorly understood.

Figure 2: The reproductive cycle of the malaria parasite



Source: Armand Gran (CRESIB)

The genome of the malaria pathogen contains over 5000 genes, each encoding a different protein. Other pathogens, for instance viruses, have very few genes, and some even encode a single toxin that is responsible for the clinical symptoms of the disease. The *Plasmodium* species are therefore very complex microorganisms. Without a thorough understanding of how the parasite functions, it is almost impossible to identify the proteins that might be its Achilles heel and to develop vaccines targeting them. It is impossible to test all of the proteins systematically, and useless to test them randomly!

Clearly, we need a better understanding of the biology of this parasite and the immune responses it provokes if we are to develop a highly effective vaccine against malaria or develop new treatments. This deeper knowledge and understanding can only be achieved through basic research.

... but we are still rummaging around inside

The Centre for International Health Research (CRESIB), ISGlobal's research centre, currently has several lines of basic scientific research focused on identifying weaknesses in the armour of the malaria parasite.

A Sweet Target

The proteins that are secreted or expressed on the surface of cells are usually bound to sugars. This process, called glycosylation, is essential to the proper functioning of these cell surface proteins.

Until now, the only glycosylated structures reported in *P falciparum* have been glycolipids that anchor certain essential proteins to the surface of the parasite and which, according to some studies, have a toxic effect on the host. However, the *Plasmodium* genome contains metabolic pathways for the synthesis in the parasite of previously unknown sugar precursors that may be involved in other, as yet undescribed, glycosylations.

In CRESIB, we are working on the reconstruction of the synthesis pathways of these precursors in order to identify new therapeutic targets: reactions that are essential to the parasite and which, if inhibited, would make it possible to eliminate them.[1]

Catch That *Plasmodium*!

One of the greatest obstacles to eliminating malaria is the ability of the causative pathogen to evolve and adapt to changes in its environment. Recent work carried out by CRESIB indicates that this ability is largely due to heritable differences in populations of genetically identical organisms,[2] a phenomenon called epigenetic variation.

In effect, research has shown that not all the parasites in a population with identical genes (twins) use the same genes. This epigenetic variation is the basic mechanism through which the parasite adjusts to natural changes in its environment and even to the presence of certain drugs or vaccines. A clear understanding of how the organism evolves and adapts to new situations is essential if we are to stay one step ahead of the parasite and develop tools to which it cannot adapt. These findings may help prioritise research and the selection of drugs and vaccines that are worthwhile taking forward to the clinical development phase, thereby saving time and money. Another line of research in CRESIB is investigating how to interfere with the parasite's ability to adapt.

Avoid Crowds

Another CRESIB team is investigating how the malaria parasite binds to molecules in the organs of the human host (for example, the placenta or the brain), allowing it to reach high densities in these sites. Accumulation of *P falciparum* in these organs gives rise to complications such as cerebral coma, severe anemia, and preterm delivery. Identification of the molecular mechanisms involved in these interactions and those of the immune responses capable of blocking such adhesions could open the door to the development of new strategies for reducing the severity of malarial infection and save millions of lives every year.[3-4]

A Change of Jacket

To understand the molecular basis of *P vivax* pathology we are studying the variant genes of this species, which play a major role in its pathology. It is difficult to study these genes because of their variability. To understand the problem, just imagine that a hotel employee is sent down to the street to identify a guest. The employee goes down, identifies the person, who is wearing a red jacket, and brings back the description. Armed with that

[1] L. Izquierdo, B.L. Schulz, J.A. Rodrigues, M.L.S. Guther, J.B. Procter, G.J. Barton, M. Aebi and M.A.J. Ferguson, 2009, "Distinct donor and aceptor specificities of Trypanosoma brucei oligosaccharyltransferases", The EMBO Journal, 28:2650-61.

[2] N. Rovira-Graells, A.P. Gupta, E. Planet, V.M. Crowley, S. Mok, L. Ribas de Pouplana, P.R. Preiser, Z. Bozdech and A. Cortés, 2012, "Transcriptional variation in the malaria parasite Plasmodium falciparum", Genome Res., 22:925-38 (destacado en Nat. Rev. Genet. 2012, 13:298-9).

[3] Bernabeu M, Lopez FJ, Ferrer M, Martin-Jaular L, Razaname A, Corradin G, Maier AG, Del Portillo HA, Fernandez-Becerra C. "Functional analysis of Plasmodium vivax VIR proteins reveals different subcellular localizations and cytoadherence to the ICAM-1 endothelial receptor". Cell Microbiol. 2012 Mar;14(3):386-400.

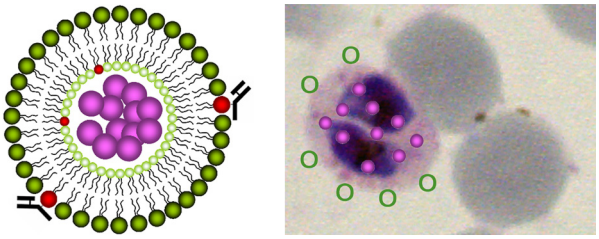
[4] Mayor A, Hafiz A, Bassat Q, Rovira-Vallbona E, Sanz S, Machevo S, Aguilar R, Cisteró P, Sigaúque B, Menéndez C, Alonso PL, Chitnis CE. "Association of severe malaria outcomes with platelet-mediated clumping and adhesion to a novel host receptor". PLoS One. 2011 Apr 29;6(4):e19422.

information, a second person is then sent to find the same visitor and bring them upstairs; however, during the intervening period, the guest has changed his red jacket for a green one. This is what happens to our immune system: each time we develop an immune response against one of these genes, the parasite changes its jacket and the whole task of identifying the pathogen has to start again from zero. Our research in CRESIB is aimed at gaining a better understanding of the mechanisms involved in these changes so that we can eventually make a definitive identification of the parasite and destroy it.

The Magic Bullet

When the malaria parasite invades red blood cells it leaves traces of its presence on the cell surface; these locks (or receptors) can be recognised by a key (or ligand). The aim of our research is to design small capsules of antimalarial drug equipped with the appropriate key. These nanovectors can be created by attaching a suitable key to a small capsule (or nanoparticle).

Fig 3. Diagram illustrating the magic bullet against malaria[5]



Source: J. Control. Release 2011

CRESIB researchers are working to build nanovectors that will deliver antimalarial drug to infected red blood cells while bypassing healthy blood cells and all other body cells, in other words: a magic bullet. If we succeed in creating such a targeted carrier, we can administer drug doses that are low enough to reduce harmful side effects but high enough to achieve the local concentrations needed to eliminate all living parasites.[6]

The Trojan Horse

In CRESIB, one of our aims is to identify new targets and develop a new platform for vaccines against *P vivax*. To do this, we are using the biology of the targeted parasite itself. *P vivax* invades immature red blood cells known as reticulocytes. To mature into red blood cells, these reticulocytes shed their membrane, forming tiny extracellular vesicles. We suspect that the parasite uses these nanovesicles to send signals to various human organs and to establish chronic infections and we have already established that they contain parasite proteins. When we isolate such nanovesicles and use them to vaccinate rodent models, they protect the mice from lethal infections.

Our current task is to identify the parasite proteins associated with such protection. We are also working on a scalable method for producing nanovesicles that could be used in a "Trojan horse" attack on the parasite. It is possible that the same model may even prove useful in combating other diseases.[7]

Perpetuating the Memory

A key issue in the design of the second generation of malaria vaccines is to characterize the protective immune responses induced by the RTS,S candidate vaccine and the factors that determine the duration of the immunity it confers. To address this question, we must once again turn to basic science. The aim of our research in CRESIB is to identify the physiological processes that must be stimulated to combat the parasite. The immune system of an individual who has received effective vaccination functions as a complex network of cells and a signalling cascade that is activated whenever the body is reinfected; it recognises the parasite and attacks it more quickly, thereby stopping the disease before it develops.

Our research is focussed on identifying the T cells, B cells and immune mediators (antibodies,8 cytokines and chemokines) found in the blood of children vaccinated with RTS,S that perpetuate the immunological memory. This knowledge would allow us to manipulate, if necessary, the induction of immune responses through improved adjuvants and formulations that would extend the duration of the protective response.

Basic scientific research is one of the fundamental pillars of medical research. It paves the way for advances needed to address the major health challenges faced by humanity. In CRESIB we are passionate in our support of basic research because we know it is an essential weapon in the fight to eradicate malaria from the face of the earth.

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[5] The green capsule on the left contains the drug (pink capsules), which is coated with molecules that recognize the red blood cells infected with Plasmodium. The diagram on the right shows the result of the magic bullet: drug is delivered only to infected cells and bypasses healthy cells.

[6] P. Urbán, J. Estelrich, A. Cortés and X. Fernàndez-Busquets, 2011, "A nanovector with complete discrimination for targeted delivery to Plasmodium falciparum-infected versus non-infected red blood cells in vitro", J. Control. Release, 151:202-11.

[7] Martin-Jaular L, Nakayasu ES, Ferrer M, Almeida IC, Del Portillo HA. "Exosomes from Plasmodium yoelii-infected reticulocytes protect mice from lethal infections". PLoS One. 2011;6(10):e26588. (Patente nº 10800764.2).

[8] Campo JJ, Dobaño C, Sacarlal J, Guinovart C, Mayor A, Angov E, Dutta S, Chitnis C, Macete E, Aponte JJ, Alonso PL. "Impact of the RTS,S malaria vaccine candidate on naturally acquired antibody responses to multiple asexual blood stage antigens". PLoS One. 2011;6(10):e25779.