



Genetic variability makes it possible for the *Plasmodium* to by pass the defenses of the human organism

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Published in November 2006

It is as if it had a thousand garments and masks. Every two days, when it is reproduced inside the red blood cells, the protozoon that causes malaria manages to generate new combinations of its genetic material and so produce extremely diversified proteins that allow it to escape from the defenses of the human organism. This capacity for genetic recombination, shown by a research group from the University of São Paulo (USP), has serious implications for the development of vaccines against this disease, because it makes them an even bigger challenge. It also makes the symptoms vary from person to person, albeit subtly, but enough to make this ailment, typical of poor countries, pass on without being detected from the first moment. The field surveys that complement the research done in the laboratory indicate that people may become resistant to some of these variations, but sensitive to others, subjecting themselves to new malarias with the same intensity as the first time.

In one of the laboratories of USP's Biomedical Sciences Institute (ICB), biologist Erika Hoffmann studied the genetic variability of MSP-2, a protein abundant on the surface membrane of *Plasmodium falciparum*, a parasite that causes the more serious forms of malaria, with convulsions and loss of conscience, besides intense fever. Her study, published in July in the *Gene* journal, was based on the blood samples taken from eight inhabitants of Ariquemes, a municipality in Rondônia where this disease was very common. As she saw, these men were infected with at least 44 different variants or strains of *Plasmodium falciparum*, which brought nine different versions of the MSP-2 protein. One of them carried nine strains, different enough to the point of behaving

like different parasites. It was an indication that both this individual as the others, to a lesser intensity, had been infected with *Plasmodium falciparum* genetically very different amongst themselves, even though found in an area with very low transmission levels. MSP-1, another protein common on the surface of the *Plasmodium* that is one of the main candidates to be a vaccine against malaria, is also much modified and thus fails to be recognized by the organism. It is as if the maze, in itself perturbing, were to spread out more and more, without Ariadne's thread to point to a way out.

Some strains of the parasite can be more aggressive than others, producing a disease of variable seriousness, or with different symptoms. Just a headache, diarrhea and dizziness may appear, instead of the shivers and intense fever that reappears every 48 hours. "At least a part of the response of the organism depends on the specific type of the strain of the parasite", comments physician Marcelo Urbano Ferreira, the coordinator of this group from the ICB. If a person had never had any contact with a strain, particularly the rarer ones, the disease tends to be more serious; if a variation that is already familiar to the organism appears, the malaria may develop – the parasites reproducing themselves initially in the liver and afterwards in the red blood cells, but without any symptoms.

"The possibility of infections emerging without symptoms, or with only some symptoms, not necessarily the most typical ones, may make the diagnosis and treatment of the malaria very difficult", Ferreira says. Another reason for which the ailment may spread more easily in silence is that normally people themselves turn to the medical services when the symptoms appear; without symptoms, they will not go to the health



MARCELO URBANO FERREIRA/USP

After the elusive protozoon: research assistant Adamílson Luís de Souza collects blood from Mercedes Andreatto da Silva, a teacher from a small rural community of Acrelândia

In contact with malaria



Doctors without frontiers: researchers visit inhabitants of rural communities and collect blood samples wherever they...

centers and therefore will not be given treatment, but they will remain infected. For this reason, they may infect the mosquitoes that can transmit malaria should they sting them in search of blood, and afterwards sting someone else. In a review published in May, José Rodrigues Coura and his team from the Oswaldo Cruz Institute of Rio de Janeiro estimated that one in four cases of malaria in Amazonia is asymptomatic and, for this reason, it makes the control of this ailment difficult.

It is estimated that 40% of the world population, equivalent to 2.4 billion persons, is exposed to infection, particularly in the tropical and subtropical regions of the planet. Every year, from 300 million to 500 million new cases of malaria arise, the most disseminated of the diseases caused by a parasite, which causes at least 1.5 million deaths, particularly of children less

than 5 years old in Africa, the most affected continent. In Brazil, the total of new cases went up from 50 thousand a year three decades ago to the level of 600 thousand cases a year, which is still the current figure. This leap is due to the opening up of roads, the construction of hydroelectric power plants, internal migration, the formation of rural settlements, and the growth of cities, which are an indication of efforts to populate the national territory. It is for this reason that malaria today is rare in the major urban centers and is concentrated in the Amazon area, where over 90% of the cases recorded in South America are found.

Varied responses - One more complication emerged from the researches of this team from the ICB: the human organism can activate different defense mechanisms in response to one strain

or another. Physician Mônica da Silva Nunes, from Ferreira's group, evaluated how one kind of defense cells, the T lymphocytes, extracted from blood samples of inhabitants from the rural zone of Acrelândia, a municipality in Acre, recognized six MSP-1 variants from the *P. vivax*, the species that currently accounts for the majority of the cases of malaria registered in Brazil and in the south and southeast of Asia. In parallel, Melissa da Silva Bastos, under the orientation of Sandra Moraes-Ávila, from the Tropical Medicine Institute of São Paulo, was investigating whether the variants of the MSP-1 induced the production of antibodies, which represent another form of defense against microorganisms. Comparing the results, they concluded that the most variable regions of the MSP-1 are those that activate the most intense responses of the organism, producing more defense cells or more antibodies. The more stable regions of this protein were those that mobilized the T lymphocytes least.

In turn, the studies under way with the MSP-2 of *Plasmodium falciparum*, carried out jointly with Kézia Scopel and Erika Braga, from the Federal University of Minas Gerais, suggest that the fact that the organism has produced antibodies against one strain of this protein does not necessarily mean that it will manage to protect itself against this strain when it appears. Another finding is that the defense system recognizes some variants, but pays almost no attention to others. "Often, a person simply fails to recognize the variant of the parasite that is infecting it", says Ferreira.

THE PROJECTS

Genome and post-genome approximation to the study of human malarías by Plasmodium vivax and P. falciparum in Brazilian Amazonia

MODALITY

Thematic Project

COORDINATOR

HERNANDO DEL PORTILLO – ICB/USP

INVESTMENT

R\$ 3.087.101,23 (FAPESP)

Acquisition of immunity against P. vivax: longitudinal study in a rural community of Amazonia

MODALITY

Regular Line of Research Grants

COORDINATOR

MARCELO URBANO FERREIRA – ICB/USP

INVESTMENT

R\$ 124.145,18 (FAPESP) and
R\$ 20.000,00 (CNPq)



NATAL SANTOS DA SILVA/USP

...have to, even in the forest. They only stop the journey, often made by motorbike, when fires break out

“Accordingly, only a part of the vast repertoire of MSP-2 variants is recognized by the immune system of people exposed to malaria in Brazil.”

And so, producing more defense cells or more antibodies, the organism will react with greater or lesser speed, in the attempt to contain the parasite, which reaches the liver 30 minutes after the sting of the transmitting mosquito. There, in the human body’s largest internal organ, after ten days, each cell generates 40 thousand others that invade the red cells that circulate through the veins and arteries. During the asexual reproduction of the parasite, which takes place inside these cells of the blood, the DNA molecule, which carries the genes, creates another copy of itself. However, the molecule that is being formed, and that ought to be identical to the original, rebels and forms a loop, which will mean that other DNA stretches are added or lost. Accordingly, the DNA copies turn out larger or smaller than the original version. And so an even greater genetic diversity is formed in the laboratory than can arise during sexual reproduction, which happens in the mosquito. Every two days, each cell of the *Plasmodium* forms from eight to 32 cells, which burst the membranes of the red cells – which is when a high fever takes place.

One of the peculiarities of this work is the close connection between the laboratory activity and the field. Mônica accompanied the reactions of the cells and antibodies to the MSP-1, working in a laboratory built in the Health Center of Acrelândia, a municipality formed from rural settlements. She moved there in

February 2004, and up until June 2005 she studied the malaria brought or acquired by the 467 inhabitants in a rural area 50 kilometers from the town. During her stay in this and in other regions of Amazonia, 63% of the inhabitants now had malaria caused by *Plasmodium vivax* and 45.8% by *P. falciparum*.

Every day, Mônica would visit the health centers after recent cases of fever, which also could be a sign of other diseases, like dengue. Soon after the rainy season, when the river is low and ponds are formed that act as a breeding ground for the transmitting mosquitoes, she would collect blood from 10 to 15 persons a day – each infection, as was to be seen shortly afterwards, caused by parasite genetically different from each other. Marcelo Ferreira, who coordinated the group and lived in Rondônia for two years, does everything possible for his pupils to get to know malaria close up.

“We can go far further in the scientific work if we don’t take malaria merely as an object for study, but as something that causes human suffering”, he says. For him, the field work could also make more original advances possible and a greater competitiveness for the Brazilian research groups, since *Plasmodium falciparum*, more common in Africa, is now adapting to the laboratory life, while *Plasmodium vivax*, predominant in Brazil, still cannot be cultivated *in vitro*.

Since 2005, it is Natal Santos da Silva, an Acrean doctor and infectologist trained in São Paulo, who has been representing USP’s team in Acrelândia. By motorcycle, he covers from 150 to 200

kilometers a day, the major part on earth roads, to find the inhabitants in the rural zone of Acrelândia who have contracted malaria. As soon as he finds them, he does examinations and collects blood samples for a month, to evaluate the effectiveness of chloroquine and primaquine, the two medicines most used against *Plasmodium vivax*, and to understand why the disease reappears after treatment, sometimes in the same month. Of the 78 inhabitants from whom he had already brought together material to study, 14 had had up to four relapses in one year: one 2 year old child, who was not included in the study but whom he attended, had already had malaria four times.

“If we manage to show a pattern of resistance of the *Plasmodium vivax*, we can propose changes in the form of treatment, or even in the medicines”, comments the doctor, who works with the support of a malaria control team of the Acre State Secretariat for Health. “It may be that the remedies are not working adequately any more, particularly in the high risk transmission areas.”

In Brazil, he reminds us, the standard dose of primaquine – used to combat the initial forms of Plasmodium, still in the liver, jointly with chloroquine, which eliminates the parasite from the blood cells – is half that recommended by the World Health Organization (WHO). Silva arrived to stay for one year, but he should remain much longer and help to create a permanent research base, forging bonds with the inhabitants and the medical services in this municipality. ■