Simian malaria models for research on human disease

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Professor and Mrs. Dissanaike, Dr. Anthonis, The Chancellor, and Professor Lakshman, the Vice Chancellor of the University of Colombo, Professor Lalitha Mendis the Acting Dean, Faculty of Medicine, Colombo, Honoured Guests and Colleagues, one of the significant contributions which Professor Dissanaike made to science is his work on simian malarias. He described 3 new species of malaria parasites with which monkeys of Ceylon are infected (1-4). Although this work constituted only a small proportion of his 117 or so publications, it probably was one of the most significant in terms of its impact. The following is an account of some of our own research on monkey malarias which followed, using the very species of parasites he described, and the monkeys in which he found them. I have chosen several subjects because of their implications on human health, although in the time available only the broadest outline of this research can be given.

Plasmodium fragile is a natural parasite of monkeys, of both the gray langurs *(Presbytis entellus* and toque monkeys *(Macaca sinica)* of Sri Lanka, which Professor Dissanaike described (3); this parasite host system is one which, in many ways resembles the system of *Plasmodium falciparum* in humans, the most dangerous malaria parasite of man. One of the reasons for saying so, is that as *P. falciparum* does in its human host, *P. fragile* too undergoes sequestration in its natural host, the toque monkey. Sequestration is the adherence of erythrocytes infected with schizonts, the mature blood stages of the parasite, to the endothelial cells lining post-capillary venules in organs such as the brain and muscle; this property of the parasite known as cytoadherence is widely believed to be the reason why *P. falciparum* malaria alone, and not the other human malarias can cause severe and complicated disease (5). Other human malarias such as *P. vivax* and *P. malariae* give rise to uncomplicated disease and do not sequester.

In 1981, long after Professor Dissanaike's period, we were using the *P. fragile*-toque monkey system to study the basis of sequestration, when we discovered and reported an entirely new phenomenon in malaria – this was that erythrocytes infected with *P. fragile* schizonts formed rosettes with uninfected erythrocytes, and that these rosettes are found transiently in the circulation of the host before they disappear to be sequestered (6). Dr. Handunnetti who was then engaged in this work while reading for her PhD subsequently showed that *P. falciparum* also formed rosettes in humans, but not *P. vivax* or any of the other non-sequestering malaria parasites (7). The discovery of rosetting was also made at about the very same time by a group of scientists in Sweden (8), both groups working independently without knowledge of each other's work. A more recent study by this Swedish group has demonstrated that parasites in patients who developed cerebral malaria in Africa formed larger and many more rosettes than those who had uncomplicated malaria (9). Rosetting is now accepted as one of the two major pathogenic properties of falciparum malaria, and laboratories elsewhere are attempting to identify the chemical nature of the molecules that are involved on the surface of the infected erythrocytes in the formation of rosettes(10).

Using the same host-parasite system Dr. Shiroma Handunnetti went on to detect antigens on the surface of infected erythrocytes, and demonstrated that, first, these antigens varied during the course of an infection; the blood

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infection of *P. fragile* in the toque monkey takes the form of waves of parasitaemia, and parasites of each wave were found to express a different variant surface antigen; we were able to type each variant antigen using variant specific immune sera which were raised in animals (11). The most significant finding of these studies was that the parasite switched antigens in a predetermined sequential manner, and this sequence was maintained in an uninterrupted manner during a single infection as well, as if parasites of any particular variant type were passaged into another host. This was the first description of the sequential expression of variant antigens in malaria (11), and it showed that antigenic variation is not the result of random mutations being selected by antibodies. After 10 years and a major effort, scientists have recently cloned these variant surface antigens of *P. falciparum;* it appears that not a single gene but a family of genes that control the expression of these and several other erythrocyte binding proteins (12-15). The significance of these studies are linked to the fact that these proteins, i.e., variant antigens, as well as the molecules that mediate rosetting, are thought to be involved in the pathology of severe and complicated falciparum malaria (5); these findings may form the basis of developing reagents such as antibodies, drugs or even vaccines which could reverse or prevent cytoadherence, and which would thus have the potential to save people from death due to severe and complicated malaria.

I will now describe a set of experiments which paved the way for another line of research that led to a better understanding of malarial pathogenesis. Just as much or more than *P. fragile* is considered the counterpart of *P. falciparum* in humans, *P. cynomolgi* is almost certainly the analogue of *P. vivax* in man; it is thought that *P. vivax* is in fact *P. cynomolgi,* which evolved separately in humans when they broke away from their simian ancestors carrying with them the monkey parasite. The subspecies ceylonensis was described by Professor Dissanaike in Ceylon monkeys (3).

When these animals are infected, the parasite numbers in the blood increase rapidly over a few days until a dramatic event occurs – this is that on a particular day, the parasitaemia declines precipitously, and on that day intraerythrocytic parasites in the circulation appear morphologically distorted. The phenomenon referred to as 'crisis' occurs particularly if the animal is splenectomized, and has been well known to scientists for years (16, 17), although its cause was unknown. In 1988 Dr. Tissa Naotunne while working for his PhD was able to identify its basis. Time does not permit me to describe the experiments, but only the findings that were made. During the rupture of schizonts, of which there are large numbers on the day of crisis, parasite exo-antigens are liberated, which stimulate blood mononuclear cells to produce two cytokines TNF-alpha and gamma interferon, each of which act in conjunction with an entity unidentified at the time and referred to as 'complementary factors', to kill parasites within erytrocytes (18). This leads to the sudden decline of the parasitaemia. We have since been able to demonstrate that one of the 'complementary factors' (in human *P. vivax* malaria) is a parasite product and that the final mediators of this parasite killing include reactive nitrogen intermediates such as nitric oxide and that the phenomenon is dependent on the presence of nucleated cells (19).

Having ascertained the immunological basis of *P. cynomolgi* 'crisis' in monkeys, we were interested to know what the analogous event to this was in human malaria, if there was one. The answer to this emerged a couple of years later in studies performed by Dr. Nadira Karunaweera while reading for her postgraduate degree. It appears that the closest analogy to crisis in monkey malaria infections, is the well known fever paroxysm in human malaria. Acute malaria infections are characterized by periodic sharp spikes of fever accompanied by chills and rigors which occur every other day, referred to as paroxysms; they correspond to the rupture of erythrocytes infected with schizonts, the mature stages of the parasite. We were able to show, that schizont rupture leads to the production by the host of the cytokine TNF-alpha, (and not gamma-interferon as in monkeys) and that, this

cytokine is an important element in the causation of fever in malaria (20). We monitored the event of a paroxysm in 9 patients having *P. vivax* malaria and in all but one of them, the sharp rise and fall of body temperature was followed by a corresponding and parallel rise and fall in plasma TNF-alpha levels (20). It is this evidence, along with a study conducted in The Gambia, in which they found subsequently, that giving an anti-TNF monoclonal antibody to African children with severe and complicated malaria resulted in a lowering of the fever (21), that implicates TNF-alpha as the pyrogen in malaria. The experimental system underlying these findings, which I did not describe due to lack of time, is now leading us to identifying the toxin(s), the chemical substance(s) in malaria which causes disease.

Finally, we have used this host-parasite system, *P. cynomolgi* in the toque monkey to test a vaccine candidate against *P. vivax* malaria for humans. MSP-1 is one of several antigens of human malarias which are potential vaccine candidates (22). Dr. Preethi Udagama working with us in 1989 first identified and described this antigen in *P. vivax* (23), and our collaborators at the Institut Pasteur cloned and expressed a recombinant antigen for vaccine studies (24). One of the biggest hurdles in developing a vaccine against malaria is its testing. It takes a tremendous effort, expense and time to develop a potential vaccine candidate up to clinical testing in humans, and until clinical trials are performed one does not know how effective it will be as a vaccine. If at this stage the trials fail, the risk seems far too great to have been taken in the first place. We have long been advocates of natural simian host-parasite systems such as the ones described by Professor Dissanaike, which are analogous of human malaria systems, as models for testing vaccine candidates for humans; our collaborators at the Institut Pasteur in Paris, cloned the MSP-1 antigen in *P. cynomolgi* (25) and formulated a vaccine construct expressed in baculovirus infected insect cells. We have just tested this *P. cynomolgi* vaccine construct in toque monkeys, the natural hosts of *P. cynomolgi ceylonensis*, by immunizing the animals with the *P. cynomolgi* constructs and challenging them with the homologous parasite. Compared to the control animals which received only saline (3 animals) or the adjuvant (Freunds adjuvent) (3 animals) all of whom developed parasitaemias which peaked at 0.6 to 3.8%, the 3 animals which received the 19 kDa vaccine construct developed either no detectable parasitaemia at all (one animal), or a very mild parasitaemia reaching values less than 0.002% which they self cured very rapidly (2 animals); thus a high degree of protection against a blood infection was achieved with this vaccine construct. Further testing is being planned with an adjuvant such as alum which is acceptable to humans, which, if successful would pave the way for human testing of a vaccine based on this construct.

I have described here, several research studies, the results of which have, or are likely to have application to human malaria and to developing tools to prevent or to treat the disease. I hope it illustrates the fact that research done, in this case by Professor Dissanaike, on what was then the trend in parasitology, describing new parasites and life cycles, had, not very long after, led to applications which have a direct bearing on human health.

Prof. Dissanaike, Sir, your work commands great respect in our minds, and it has been a great source of inspiration to us. We believe that the most fitting tribute to the high standards you set, and to your contributions to science, is to continue research ourselves, and I hope we've been successful in this. To all of us at the Department of Parasitology and the Malaria Research Unit it is a great occasion to be able to felicitate you. I wish for you and Mrs. Dissanaike, whose gracious support must have been essential for your achievements, a long, healthy and happy life.

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