P. B. Fernando Memorial Oration - 1994

The pathogenesis of human malaria

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The President, College of Physicians, The chief guest of the Annual Sessions Professor Sir, David Weatherall, Other distinguished foreign physicians, the family and friends of the late Prof. P. B. Fernando, Fellows and Members of the College of Physicians, ladies and gentlemen, and colleagues, the late Prof. P. B. Fernando in whose memory this oration is being delivered was an eminent physician of Sri Lanka, then Ceylon; I feel much honoured to have been asked to deliver this oration.

Professor Fernando qualified in Ceylon as a Licentiate in Medicine and Surgery in 1924 and served as a Medical officer in various parts of the country. He did his postgradute studies in Britian, obtaining the Membership of the Royal College of Physicians of London in 1932, and soon after, the Diploma in Tropical Medicine and Hygiene. Upon returning to Sri Lanka in 1933 he was appointed Visiting Physician to the General Hospital Colombo, and in 1936 he became the first Professor of Medicine of the Ceylon Medical College. When the University of Ceylon was established in 1942 he was appointed to the chair of Medicine. He held several other positions concurrently, as visitng Paediatrician to the Lady Ridgeway hospital, and acting on occasions as Dean of the Faculty of Medicine and the Vice-Chancellor of the University of Colombo.

He had been an outstanding clinician, a keen researcher and a very good teacher with a meticulously thorough approach to his work, an eye for detail. His research pulications in paediatrics encompass tuberculosis, ascariasis and nutritional disorders. He had an interest in anaemia particularly in erythroblastosis foetails, and in general medicine he made an outstanding contribution through a paper titled "A clinical study of 647 patients treated during the malaria epidemic in Ceylon in 1934". In 1939 he published and contributed knowledge that rehumatic infection was an important cause of heart disease in Ceylon. He also studied ischaemic heart disease in relation to atherosclerosis, and incriminated coconut oil in the diet, as an important atherogenic agent in the aetiology is ischaemic heart disease, particularly in persons in lower income groups. The undergradute teaching programme in Medicine at the University of Colombo had come to be of excellent repute under his direction. Aove all, it is reported that he was a kind and compassionate person, quite incapable of uttering a harsh word; he was, not suprisingly a quitely practicing buddhist.

Had he lived today, one wonders, what he would have thought of the world of medicine as it is practised and researched now. Many changes have occurred in medicine since his time, and indeed in the world at large, both scientifically, and in terms of knowledge and attitudes. I believe, that he would have approved of the practice of medicine in an honest and dedicated manner, and that he would have subscirbed to a rigorous intellectual pursuit in medical research. I fervently hope that the attributes are even remortely reflected in the work that I will describe to you shortly, which will form the subject of this oration. This oration is meant to provide a glimpse into one of the areas of research that my colleagues and I have undertaken, on a human disease which is important to Sri Lanka and the tropical world at large, the disease being malaria. I would first like to place the disease in perspective.

Malaria in Sri Lanka causes an illness which in most patients, leads to acute suffering and considerable ill health[1]. In most patients in Sri Lanka the disease runs an uncomplicated course, implying that the infection is not life-theratening, leading to recovery without apparent sequalae. Even such infections, we estimate, incapacitates a person for 14 days on average, despite seeking early treatment which is the norm in Sri Lanka. Despite years of study and ensuing knowledge, we still probably underestimate the effect of malaria on people who live in endemic areas and suffer repeated infections during a life time; its effect on cognitive and psychosocial development is largely unknown. The severe and complicated forms of malaria such as cerebral malaria, and organ failures, are relatively rare in Sri Lanka but is more prevalent in other tropical countries such as Africa and the far east. About 10-20% of such infections are fatal.

Last year we held, as an official estimate, close to a 400,000 malaria infections in Sri Lanka[2], the country's population being 16 million. Malaria is a major contributor to the burden of ill health in Sri Lanka, and to morbidity and mortality in the entire tropical world.

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The recent history of malaria in Sri Lanka is a turbulent and varied one. Following a massive epidemic of falciparum malaria which occurred in the 1930's, and which reportedly killed 80,000 Lankans then, the disease was almost eradicated in the 1960s[3], a concsiderable achievement, made mainly by the use of two potent control measures — one, a powerful insecticide DDT which was effective against the mosquito that trasmits malaria. The other, a once potent drug, chloroquine, with which patients were treated. This major acheivement of near eradication, was, however, short-lived, and now, like in many other parts of the world where malaria is endemic, the disease is on the increase in Sri Lanka, with apparently poorer prospects for its control in the immediate future.

About a third (or in some areas half) of all the malaria infections being reported in Sri Lanka today are due to *P. falciparum*[2,4] the most dangerous of the human malaria parasites, which was once very rare in this country. The difficulties in controlling the disease today, are due mainly to a fast growing resistance of the malaria parasite to anti-malarial drugs. Since its emergence in 1965, extent of chloroquine resistance in the world increased dramatically to encompass almost all of the tropical world a mere 20 years later[5]. At the Thai Kampuchian border, where possibly the worst problems are encountered, about 50% of the infections are resistant to even the last alternative drug available, Quinine used alone[6].

In Sri Lanka, Chloroquine resistance in *P.falciparum* first reported in 1985, has steadily incresed, and estimates now, are that 50-60% of *P. falciparum* infections are resistant [Unpublished information]. The second line of drug Sulfadoxine/pyrimethamine is now widely in use in Sri Lanka for the treatment of *P. facliparum* infections; although it is generally effective, we have just reported the first[7], and detected the second case of SP resistance in the country [Unpublished]. Even *P. vivax* which accounts for half or more of the infections in Sri Lanka has developed resistance to chloroquine in many parts of the world [8] and it would be only a matter of time before it appears here in Sri Lanka. Insecticide resistance on the part of the mosquito vectors of malaria is quite prevalent, and newer insecticides are few and more expensive.

Research towards the development of alternative solutions for malaria control is being ardently pursued: These include molecular studies to identify new targets for anti-malarial drugs and vaccines, and to genetically engineer mosquitoes to make them less effective as vectors. Behavioural studies on susceptible human populations to reduce the risk of contracting the disease are also being undertaken[9].

I plan to speak on some of our own work which has led to a better understanding of the causation of clinical disease in malaria, i.e., the pathogenesis of malaria.

Malaria is endemic in the dry zone of Sri Lanka which comprises about two thirds of the country. In Kataragama, in the deep south of the malaria endemic zone, is our field station[4], where, we the scientists of the Malaria Research Unit, have been conducting very detailed studies on malaria in a population of about 5000, resident in several villages. These studies were detailed to the extent that we have a knowledge of exactly who in this community got malaria during the past 7 years or so, when and where, and what their disease was like. The area is typical of the dry zone, with a arid climate and seasonal rainfall. The residents are relatively impoverished peasant farming communities living in houses with a wide range of construction types, ranging from shacks with barely intact roofs, to those constructed with brick and mortar with tiled roofs. We found that in residents of the very poorly constructed houses the risk of contracting malaria was up to three times or more greater than those who live in better constructed houses[10]. The inoculation rates of malaria in this area are such, that, on average every person gets about one malaria infection per year [Goonewardena et al., in preparation for publication). In reality, however, malaria is clustered in certain individuals and households, some developed repeated infections in a year, and others none at all.

We have also had the opportunity of studying malaria in Colombo. Malaria transmisson does not occur in Colombo and its surburbs. Malaria patients are however admitted to the General Hospital in Colombo, these being adults who had contracted the disease during travel to an endemic area of Sri Lanka[11]; many of these patients would not have experienced malaria previously. These patients in Colombo are therefore non-immune with respect to malaria. If one looks at the disease profile of these Colombo patients, as we did, in 50 adults, one sees that they suffer clinical symptoms of considerable severity. We used for this purpose, a clinical assessment system developed by us to quantify the symptoms which occur during a malaria infection; the outcome of this assessment was a numerical value designated as a clinical score the higher it being the more severe being the intensity of symptoms[15].

In Kataragama, the malaria endemic region I referred to earlier, by the time people reach adulthood, they would have experienced many malaria infections, and it is generally known that they suffer less severe symptoms during an infection. The clinical score of 50 endemic patients matched on age with those of the Colombo patients, was significantly lower than those of the Colo-

mbo patients, demostrating that they did indeed have much milder symptoms during a malaria infection. We also used more objective assessments than the clinical score to measure disease, these being a large number of haematological parameters that are known to be deranged during a malaria infection; they included the eythrocyte sedimentation rate (ESR), serum bilirubin levels, cell counts in preipheral blood and haemoglobin levels. Almost all these parameters were altered to a significantly higher degree in the Colombo patients than in the endemic ones. These findings imply that not only the clinical diesease, but the underlying pathology too, was less in the endemic patients. Patients in Kataragama, had, therefore, by experiencing multiple malaria infections, acquired a degree of tolerance to the infection, which we refer to as immunity againat the clinical disease of malaria[15].

The patients in Kataragama, we found, also fewer number of parasites in thier blood, implying that they had also acquired an anti-parasite immunity. One might think that they had less symptomas because they had less parasities. This, however, was not the case because at any given parasite density, the disease severity of Kataragama patients were found to be much less than those of Colombo patients[15]. These observations imply that immunity to clinical disease can be achieved independently of immunity to parasites, a fact has been known through anecdotal evidence from Africa. African children who are much more exposed to malaria than those in Sri Lanka, and who, it is said, can be found well enough to be playing football, while having densities of malaria parasites in thier blood which in most circumstances will be compatible with severe disease. Apart from such evidence, the evidence reported here represents the first to quantify the clinical disease and the phenomenon of clinical immunity to show the distinction between anti-parasite and anti-disease immunity. If, as we have shown here, immunity to disease can be achieved independently of parasite numbers, the question then arises, as to what the basis of disease is.

To address this question let me return to the non-immune patients in Colombo who suffer a full-blown disease. One of the early observations made in our laboratory by a young student was that *P. vivax* malaria patients who were quite infectious to mosquitoes, ceased, quite abruptly, to be infective at the time of malarial paroxysms, these being episodes of fever accompanied by chills and rigors which characterize acute malaria infections[12]. Features of a typical paroxysm in one of many patients in whom we studied this is given here: At the beginning of a paroxysm is a sharp rise of fever which reaches up to a 105°F, during the early period of which the patient experiences a chill, followed by a rigor. The fever declines as rapidly as it rose, accompained by a period of intense sweating. The entire episode takes from 5-8 hours. Such paroxysms occur periodically once every 48 hours and they coincide with the rupture of asexual blood stage schizonts in the patient's circulation, which takes 48 hours to develop.

We were able to show that the sudden loss of infectivity of the malaria parasite to mosquitoes at the peak of a paroxysm was due to the appearance in the patient's plasma of substances which were either lethal to, or inactivated, gametocytes, the parasite stages that were infectious to the mosquitoes[12]. We demonstrated this by taking normal heathly gametocytes from infectious patients, incubating them for 3 hours in plasma taken from a malarial patient during a paroxysm and showing that they completely lost infectivity to mosquitoes: this was in contrast to gametocytes incubated in normal serum, which were highly infectious[12]. This parasite killing effect in paroxysm plasma was very transient in that plasma taken from the same patient 4 hours after the peak of a paroxysm had no such parasite killing effects. We were then able to demonstrate that this parasite killing activity in patient plasma was due to the presence of the cytokine TNF-alpha at the peak of a paroxysm; the addition of anti-TNF antibodies to paroxysm plasma completely abolished the parasite killing activtiy, indicating that TNF was essential for the killing. However, recombinant TNF when added to normal human plasma was incapable of killing parasites[12]. This meant that other factors, which were also present transiently in the patient's plasma, which we termed "complementary" factors were necessary for the killing, and that the TNF was acting in conjunction with them. We were subsequently able to show that one of these complementary factors is a parasite product liberated during schizont rupture[16]. Further studies led us to demonstrate that the final mediator in this parasite killing was, at least in part, reactive nitrogen intermediates such nitric oxide[17].

At the time to our surprise, this of parasite killing was almost undetectable in the plasma of patients from Kataragama who suffered little disease a malarial infection. The plasma TNF levels in the sera of these endemic patients were also correspondingly lower than in patients who were residents of Colombo[12]. These observations led us to postulate that serum cytokines are associated with clinical symptoms to the extent that 1) their transient presence in serum conicides closely with marked clinical features of malarial infections, i.e., paroxysm and 2) levels of serum cytokines are high in clinically non immune patients who experience severe clinical disease and are much lower in clinically semi-immune patients. This evi-

dence suggested that serum cytokines may be involved in the causation of clinical disease in malarial infections.

We subsequently provided more evidence to implicate TNF in the cauastion, specifically, of malarial paroxysms. Paroxysms were closely monitored by us in 14 acute *P. vivax* patients, in 9 of whom we also monitored changes in plasma TNF. In all but one of these patients a remarkerble parallel was found between the sharp rise and fall in body temperature and plasma TNF levels[13]. The dynamics of the rise and fall in TNF levels closely followed the dynamics of temperature change. It is this evidence, together with the results of a clinical trial of an anti-TNF antibody carried out in severely ill *P. falciparum*-infected Gambian children, by Kwiatkowski and his coworkers, which implicates TNF in the causation of fever in malaria[18].

Mature schizonts of the infected erthrocyte, and during this rupture, release parasite exo-antigens into the circulation. These parasite exo-antigens then stimulate peripheral blood mononuclear cell to produce TNF and possibly other complementary parasite killing factors.

Because clinically immune individuals have lower levels of cytokines and other parasite killing factors, it appears that clinical immunity to malaria is achieved by maintaining low levels of these cytokines, and/or the other factors in the serum. We were able to demonstrate at least three mechanisms by which such a state could be achieved[14]. One is that the cell of such clinically immune patients are down-regulated in thier response to malaria toxins. We have shown this by taking peripheral blood cell from clinically immune persons and stimulating them with bacterial lipopolysaccharide and demonstrating that they produce significantly less amounts of TNF compared to cells form non-immune persons from Colombo[14]. The mechanism underlying the down-regulation of these cells is quite unknown. Secondly, we have demostrated that clinically immune malaria patients have antibodies in their sera, to the malaria toxin(s). Their serum is able to suppress the induction of TNF by cells when stimulated by the toxin[14]. Finally we have recent evidence which suggests that clinically immune patients may be tolerant to TNF; we found that at any given level of plasma TNF, endemic patients experienced much less disease (as assessed by the clinical score) than patients in Colombo[14]. Here too, the mechanism of TNF tolerance in man is still unknown; tolerance to TNF could be mediated via TNF-receptor expression, or by the acquisition of antibodies to TNF.

The evidence I have presented here implicates cytokines among the mediators of disease, and malaria toxins which are liberated during schizont rupture, in their induction. Up to his point in the lecture, it is the clinical disease in uncomplicated malaria infections that has been addressed.

TNF-alpa has also been implicated in severe and complicated malaria, a rare form of the disease which threatens life. Several investigators have shown that the severity of *P. falciparum* infections in children correlate broadly with plasma TNF levels[19,20,21]. The search has now begun for the malaria toxin(s), with markers now being available for the toxin activity. TNF inducation, and perphaps more appropriately, parasite killing are two markers of the toxin activity which have emerged from recent studies[16,22].

Detailed studies on *P. vivax* paroxysms have led us to identifying several other biological phenomena which are closely linked to paroxysms. One such event is a marked increase in peripheral circulation, of T-lymphocytes bearing the gamma-delta phenotype during the period of a paroxysm. As we have recently shown[23], these gamma-delta T-cells which normally constitute less than 3-5% of peripheral blood mononuclear cells, show a significant increase in numbers during paroxysms in non-immune patients, when they have been found to constitute up to 30% of peripheral blood mononuclear cells. These cellular changes are consistently absent in the clinically immune, even during a paroxysm[23]. It appears that this increase in gamma-delta T-cells in the peripheral circulation represent a rapid proliferation induced by malaria toxins; we have preliminary evidence that plasma taken from acute non-immune malaria patients can induce the rapid proliferation of these cells obtained from a non-malarial individual when they were cultured in vitro in the presence of paroxysm plasma [Kumaradharmasena et al., unpublished]. We are, at this stage, unaware of the purpose or specific function of these cells, whether they are associated with pathogenesis or are protective to the host.

Yet another paroxysm-associated phenomenon we have demonstrated is that in the presence of paroxysm plasma, human endothelial cells are induced to upregulate the expression of receptors for cytoadherence such as ICAM-1 and CD36 [Handunnetti, Pathirana et al., unpublished]. Other workers have described the induction of hypoglycaemia by a malaria toxin[24].

Attempts are being directed towards identifying the malaria toxin(s). The hope is that if the malarial toxin and the cascade of events leading to malarial pathogenesis and indeed disability and death are known, that we may be able to intervene with therapeutic agents such as antibodies, drugs, or vaccines to alleviate morbidity in malaria[25].

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