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THE ROLE OF CYTOKINES IN *PLASMODIUM VIVAX* MALARIA

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The cytokine tumor necrosis factor and other as yet unidentified factor(s) which together mediate the killing of intraerythrocytic malaria parasites are transiently elevated in sera during paroxysms in human Plasmodium vivax *infections in non-immunes. These factors which included TNF and parasite killing factor(s) are associated with the clinical disease in malaria to the extent that their transient presence in infection sera coincided with paroxysms, the the most pronounced clinical disturbances of* P. vivax *malaria and secondly because their levels were markedly lower in paroxysm sera of semi-immune patients who were resident of an endemic area. Further, a close parallel was obtained between serum TNF levels and changes in body temperature that occur during a* P. vivax *paroxysm in non-immune patients, suggesting a causative role for TNF in the fever in malaria.* P. vivax *rarely if ever cause complicated clinical syndromes. Nevertheles serum TNF levels reached in acutely ill* P. vivax *patients were as high as in patients suffering from cerebral complications of* P. falciparum *malaria as reported in studies from the Gambia. Cytokine profiles and other changes accompanying clinical disease in* P. vivax *and* P. falciparum *malaria are compared in this paper with a view to discussing the potential role of cytokines in the causation of disease in malaria.*

Key words: tumor necrosis factor – malaria – paroxysms – gametocytes – clinical immunity

The absence of a direct correlation between the disease in malaria and presence or density of blood parasites was known to early malariologists (Sinton et al., 1931; Hill et al., 1943). In regions of hyperendemic malaria such as in areas of tropical Africa, it is children who suffer most from malaria; they have high parasitaemias and suffer severe clinical disease. However, following repeated exposure to malaria, those who survive gradually acquire an immunity such that adults come to have a lower parasitaemia and hardly suffer clinical symptoms (Sinton et al., 1931; Hill et al., 1943; Mc Gregor et al., 1956). The absence of symptoms in immune adults may not be entirely due to low parasitaemias, because older children can be seen who continue to have high parasitaemias without being clinically ill, a state which in the early literature was referred to as 'tolerance' (Hill et al., 1943). This implies that individuals may acquire an immunity to the disease before acquiring an anti-parasite immunity. It also implies a distinction between immunity to disease and anti-parasite immunity. Accumulating evidence suggests that the causation of disease in malaria may be linked to cytokines such as Tumor Necrosis Factor (TNF) that are produced during human infections with both *Plasmodium falciparum* (Clark, 1987; Clark et al., 1987) and *P. vivax* (Mendis et al., 1990), the two major malaria pathogens of man. This paper describes the evidence for a role of cytokines in disease in *P. vivax* malaria; a comparative analysis of the cytokine profiles in human *P. vivax* and *P. falciparum* infections, which have markedly different clinical features and underlying pathology, is presented here with the aim of deducing their possible role in disease.

In acute *P. vivax* infections in non-immune Sri Lankan patients a marked elevation of serum TNF occurs during paroxysms (Mendis et al., 1990); Karunaweera et al., submitted) Paroxysms are episodes of fever accompanied by chills and rigors which occur once in every 48 h. coinciding with the rupture of asexual blood stage schizonts. During *P. vivax* paroxysms there is also a transient but pronounced drop in the infectivity of parasites (gametocytes) to

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mosquitoes. We have shown that the loss of infectivity during paroxysms is due to the killing of circulating intra-erythrocytic gametocytes mediated by TNF acting in conjunction with an as yet unidentified serum factor(s) transiently present in the serum. The incubation *in vitro* of healthy infectious gametocytes in serum taken during a paroxysm (paroxysm serum) for 3 h abolished infectivity; such effects were not seen in serum taken 3 h after the paroxysm (post-paroxysm serum). The gametocyte killing effects of paroxysm serum could be abolished by the addition of a polyclonal antibody against human TNF and restored by reconstitution with recombinant human TNF (rTNF). rTNF by itself however, had no gametocyte killing activity when added to normal human serum indicating that another factor(s) which was present in paroxysm serum was essential for parasite killing. The identity of this complementary factor is yet unknown; its presence in serum must be very transient since addition of rTFN to serum taken 3 h after a paroxysm did not confer killing activity to it. Serum TNF is sharply, but transiently, elevated to produce peak levels of up to 3000 pg/ml at the time of a paroxysm. In most, but not all, of 20 patients studied serum TNF levels declined to less than 15 pg/ml 3 h later. Even in patients in whom high TNF levels were sustained 3 h later the gametocyte killing activity of their serum was completely lost implying that it was the loss of the complementary factor(s), and not of TNF, that was responsible for the loss of gametocyte killing effects.

In contrast to these marked TNF-dependent gametocyte killing effects in the sera of clinically non-immune patients, paroxysm sera of clinically semi-immune subjects from a malaria endemic region of Sri Lanka was found to be non-lethal to gametocytes. Their serum TNF levels were correspondingly lower, and declined rapidly to undetectable levels 3 h after a paroxysm.

Thus, serum cytokines (TNF) and complementary parasites killing factor(s) appear to be associated with the clinical disease in *P. vivax* malaria to the extend that (1) their transient presence in serum coincides very closely with the marked clinical feature i.e., paroxysm and (2) these serum cytokine levels are high in clinically non immune patients who experience severe clinical disease and are much lower in clinically semi-immune patients.

It has been shown that in human *P. falciparum* infections, the rupture of schizont-infected erythrocytes during schizogony leads to the release of parasite exoantigens which then stimulate peripheral blood mononuclear cells to produce TNF (Scuderi et al., 1986; Kwiatkowski et al., 1989). In *P. vivax* infections this is supported by *in vitro* studies in which parasite antigens from schizonts were added to culture of human PBMC (Karunaweera et al., submitted). Supernatants from such cultures taken 48 h later had potent parasite killing effect(s). Further, as in the case of human paroxysm serum, the parasite killing effects of culture supernatants were abolished by anti-TNF antibodies and restored by the addition of r human TNF, indicating that antigen stimulated human PBMC produce both TNF and complementary killing factor(s) in vitro.

This *in vitro* replication of the events that occur *in vivo* during a paroxysm allowed us to investigate how clinically immune patients maintain low serum levels of TNF and the complementary factor(s): the addition of convalescent serum from clinically immune endemic patients but not of the non-endemic patients effectively suppressed the production of parasite killing factor(s) by *P. vivax* exo-antigens (Karunaweera et al., submitted). This might have been achieved by the neutralization of parasite exo-antigens by serum antibodies. These studies led us to propose first, that clinical immunity to malaria is achieved by maintaining low levels of serum cytokines and other parasite killing factor(s), and secondly, that one of the mechanisms by which clinical immunity is achieved is by the acquisition of antibodies which neutralize disease-inducing parasite exo-antigens.

A link between cytokines such as TNF and disease in malaria was originally suggested by Clark (1978), and several subsequent studies of *P. falciparum* malaria have provided supportive evidence (Kern et al., 1989; Grau et al., 1989; Kwiatkowski et al., 1990). Cytokines have also been assigned a role in parasite killing during a malaria infection (reviewed by Clark, 1987; Mendis et al., 1990; Karanaweera et al., submitted). Thus opposing functions, one, of deleterious consequences (e.g., disease and even death due to complicated *P. falciparum* malaria) and the other, beneficial (by their anti-parasite activity) to the host, have to be contended with in a role for cytokines in malaria.

It seems clear nevertheless, that with increasing exposure to malaria, the host is bestowed with mechanisms by means of which cytokines are maintained at low levels for the purpose of achieving clinical tolerance; this is at the expense of losing a potential host defense mechanism against parasites. This must surely imply that with increasing exposure to malaria, cytokine mediated anti-parasite effects are dispensed with as a mechanism of natural host defense against the parasite. Since with increasing exposure the host also becomes more efficient in controlling parasite densities it is likely that the function of cytokines in parasite killing is replaced by other more specific anti-parasite immune mechanism.

The clinical disease in malaria encompasses many different forms, being the likely the outcome of distinct pathological entities. The disease typically caused by *P. vivax* is characterized by clinical paroxysm and constitutional disturbances, but rarely if ever death by itself. In contrast, in *P. falciparum* the disease ranges from a clinical form which is even milder than *P. vivax* to severe complicated malaria, the best known of which is cerebral malaria, which in a minority of cases leads to death. It is still not quite clear as to which of these clinical phenomena cytokines are be associated with; recent clinical studies however, have added much to our knowledge.

In a recent study we monitored serum TNF levels in relation to the clinical features (chills, rigors and fever) of a *P. vivax* paroxysm in which the rapid elevation of body temperature returns to near normal levels within 4-8 h (Karunaweera et al., manuscript in preparation). In 8 out of 9 non-immune patients the dynamics of change of TNF levels closely followed the dynamics of temperature change, the rise and fall in TNF appearing to proceed that of temperature by 30-45 min. Further supporting an association between the fever in malaria and TNF, a recent clinical trial of an anti-TNF monoclonal antibody on *P. falciparum* infected children in the Gambia, resulted in a significant reduction of fever in the patients who received anti-TNF therapy compared to controls (Kwiatkowski et al., submitted). These two studies present sufficient grounds to suppose that TNF is one of the principal factors responsible for the fever in malaria. With regard to the pathogenesis of severe and complicated falciparum malaria however the role of TNF is not well understood.

A causative relationship between TNF and severe complicated human *P. falciparum* malaria has been suggested from several recent studies (Scuderi et al, 1986; Grau et al., 1989; Kwiatkowski et al., 1990). In 2 of these studies (Grau et al., 1989; Kwiatkowski et al., 1990) a significant association was found between serum TNF levels and the severity and fatality of *P. falciparum* infections. The average circulating TNF levels were significantly higher in the minority of patients who had cerebral malaria compared to those who had fever without complications; further, high TNF was found to be a better predictor of a fatal outcome in these cerebral malaria cases. In both studies however, there was a considerable overlap in the serum TNF levels of children belonging to the three groups of pathology. This evidence strongly implies, but does not necessarily prove, a causative relationship between TNF and disease severity. Even a recent pilot clinical trial with a murine monoclonal anti-TNF antibody in children afflicted with cerebral malaria failed to resolve the question because the study proved to be inconclusive (Kwiatkoski et al., submitted).

If TNF is indeed playing a role in the pathogenesis of severe and complicated malaria and not merely being a marker of disease severity in *P. falciparum* it is noteworthy that in *P. vivax* infections which are uniformly non-lethal, serum TNF levels (measured at random during an infection) are as high as or even higher than those in *P. falciparum* infections with cerebral complications (Karunaweera et al., submitted; manuscript in preparation). Indeed the peak concentrations of serum TNF measured during paroxysms in *P. vivax* infections far exceeded those measured in *P. falciparum* infections, including those with cerebral malaria leading to death. Thus high serum TNF levels cannot, in themselves, be directly responsible for the symptoms unique to cerebral and lethal *P. falciparum* malaria. They may, however, do so in conjunction with other unique properties of the parasites of the parasite which are not associated with *P. vivax*. One such property is sequestration of parasite infected erythrocytes in the post-capillary venules of internal organs including those of the brain a feature characteristic of *P. falciparum* but not *P. vivax*. Parasite sequestration is widely believed to be associated with complications of *P. falciparum* malaria; experimental evidence has indeed been presented to show that TNF upregulates the expression of

parasite receptors on host endothelial cells such as ICAM-1 (Berendt et al., 1989).

Other hypotheses for mechanisms through which TNF might mediate severe and complicated malaria have been presented. One is that TNF trigger cytokine cascades that cause the release of free-oxygen radicals or nitric oxide which in turn damage cerebral tissues. In a recent paper, Clark et al. (1991) make a convincing argument for attributing cerebral pathology to nitric oxide which is released from endothelial cells following TNF stimulation. The central mechanism postulated is a highly localized derangement of neurological function through false neurotransmitter signals generated by non-neuronal NO. In this he presents an attractive proposition which accommodates most if not all of many different phenomena such as cytoadherent properties of the parasite, raised intra-cranial pressure, and elevated TNF levels which have been considered as being causatively linked to cerebral malaria.

Yet another mechanism through which TNF is considered to cause complicated malaria is through its metabolic effects of inducing hypoglycaemia and lactic acidosis. In all the clinical studies mentioned above hypoglycaemia also correlated with the severity of *P. falciparum* infection (Grau et al., 1989; Kwiatkoski et al., 1990). Hyploglycaemia however is not a feature of *P. vivax* malaria (Karunaweera et al., in preparation for publication). In view of the marked elevation of serum TNF levels which occurs in *P. vivax* malaria its is doubtful whether the hypoglycaemia seen in *P. falciparum* infections, particularly in complicated cases is at all due to TNF as has been widely supposed.

Severe and complicated *P. falciparum* malaria, which occurs in a minority of infections, bears the highest morbidity and thus presents the greatest challenge for alleviating disease in malaria. From the arguments presented above, it follows that comparative studies on cytokine mediated phatology in the two diseases, *P. vivax* and *P. falciparum*, could provide clues to possible cytokine mediated mechanism in the pathogenesis of this important clinical entity.

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