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Dynamics of fever and serum levels of tumor necrosis factor are closely associated during clinical paroxysms in *Plasmodium vivax* malaria

(paroxysm/pathogenesis/cytokine)

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ABSTRACT **Paroxysms are sharp episodes of high fever accompanied by chills and rigors that occur periodically, once in every 48 hr in *Plasmodium vivax* infections. We have measured the changing levels of serum tumor necrosis factor (TNF) during paroxysms in non-immune patients infected with *P. vivax* malaria. The changes in TNF levels closely paralleled the rise and fall in temperature during the paroxysms but tended to precede them by 30–60 min. These observations suggest that the rise and fall in temperature during *P. vivax* paroxysm may be directly related to the periodic changes in TNF levels induced during these infections. The peak TNF levels reached during *P. vivax* infections were much higher than even those which have been recorded during severe and fatal *P. falciparum* infections in which TNF has been postulated to contribute to the severe manifestations of this disease.**

The most characteristic clinical features of malarial infections are the acute paroxysmal and febrile episodes that typically recur at intervals of 48 hr in infections due to the two main human malaria parasites, *Plasmodium vivax* and *Plasmodium falciparum*. The detailed symptoms associated with these periodic paroxysms, including their severity and threat to life, are specific to the infections by each species of parasite (1). In infections of *P. falciparum*, paroxysms are typically poorly delineated and may extend irregularly over >24 hr; severe complications including cerebral involvement and coma may arise in nonimmune individuals, often leading to death. Infections of *P. vivax* in nonimmune patients are very rarely fatal and do not involve the severe complications of *P. falciparum* infections. Compared with those in *P. falciparum* infections, paroxysms in *P. vivax* infections are generally short and sharply delineated within a period of usually <8 hr. The feature most consistently associated with infections by either parasite is a sharp rise in temperature at the onset of a paroxysm, reaching peak values of up to about 41°C within the first 1–2 hr from onset.

There has been some experimental observation and much speculation on the involvement of cytokines and especially tumor necrosis factor (TNF) in the symptoms and effects of malarial infections (2–4). Speculation on the possible role of TNF in the severe complications of *P. falciparum* infections, particularly cerebral malaria, rests mainly on the evidence of a strong correlation between serum TNF levels and the severity of disease and mortality in *P. falciparum*-infected children (5, 6). Evidence has also been presented for TNF acting as a mediator of malarial fever (7, 8). Here we report observations of serum TNF changes during febrile paroxysms in *P. vivax* malaria. Our results demonstrate high TNF levels without any cerebral involvement or lethality associated with *P. falciparum* infections. They also show that the rise and fall in serum TNF levels closely parallel but slightly precede the rise and fall of temperature during a paroxysm in an infection of *P. vivax* malaria.

MATERIALS AND METHODS

Patients. The features of a paroxysm and serum TNF levels were monitored in nine patients with *P. vivax* infections. All patients were adults attending the General Hospital Colombo and were residents of regions in which malaria was nonendemic (9). Patients had acquired the infections following travel to a malaria-endemic region of Sri Lanka. All except one had no previous recorded malaria infections. Following identification of infection by blood smear, informed volunteers completed a single paroxysm without drug treatment. This was ethically justified because *P. vivax* infection is neither life-threatening nor associated with severe morbidity.

In 25 other adult *P. vivax* patients who were also admitted to the General Hospital Colombo, TNF levels were assayed soon after the diagnosis was confirmed; their paroxysms were not monitored.

Recording of Clinical Symptoms. During the entire period of a paroxysm each patient was kept under continuous observation. Oral temperatures were taken at frequent intervals and associated signs and symptoms—i.e., feeling of cold (chill), shivering (rigor), feeling of hotness, and sweating—were recorded. The time that each symptom began and ended was determined by close questioning and observation.

Bleeding. Venous blood samples (1 ml) were collected at intervals during the course of a paroxysm. Blood was collected into 0.1% EDTA containing a protease inhibitor (aprotinin, at 0.6 trypsin inhibitor unit/ml) and centrifuged at 12,000 × *g* for 10 min, and the plasma was frozen immediately and stored at −20°C for TNF assay.

Assay for TNF. TNF was measured by the immunoradiometric assay previously described (5). The kits used in this assay were kindly provided by Medgenix, Fleurus, Belgium.

RESULTS

Nine patients undergoing paroxysm of *P. vivax* malaria were monitored for fever and other symptoms, and their TNF levels were measured in blood samples taken during the episode. The paroxysms in eight of the nine patients (Fig. 1) followed an extremely regular and predictable sequence of symptoms. The first symptom was the experience of a chill

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Abbreviation: TNF, tumor necrosis factor.

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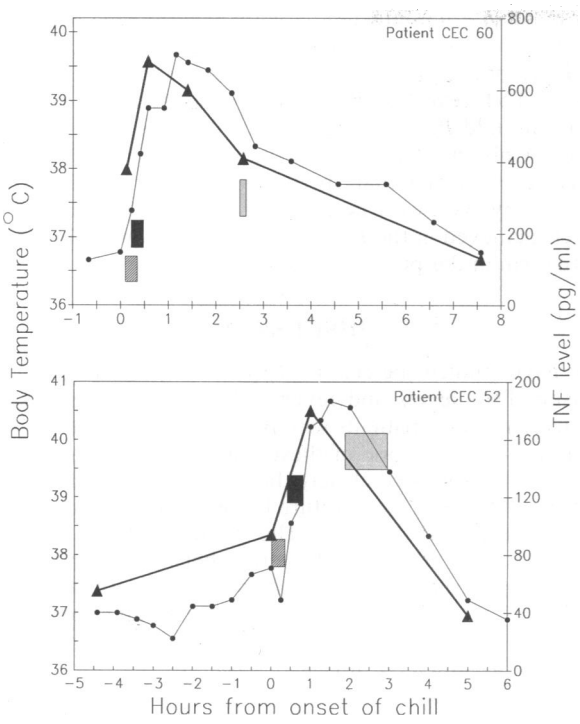

FIG. 1. Body temperature (measured orally) (●), plasma TNF level (▲), and the period and duration of the chill (hatched box), rigor (black box), and sweating (gray box) during the course of a single paroxysm in two of the nine *P. vivax* patients studied. The parallel between changes in body temperature and plasma TNF levels was as close as is shown here in eight of the nine patients studied.

that lasted between 10 min and 1 hr. Onset of the chill was followed within minutes by the beginning of the rise in temperature; rigor, with violent shivering and chattering of teeth followed, usually within 10 min after the beginning of the chill. The rigor continued usually for 10–30 min but in some patients lasted for up to 1 hr. Throughout these symptoms the temperature continued to rise, reaching a peak of 39.4–41.1°C between 1 and 2 hr after the onset of chill. At this point the patient was hot and dry and the rigors had ceased. As the temperature began to fall a period of profuse sweating followed, which lasted between 10 min and 1 hr. Compared with the rapid rise in temperature over the first hour of the paroxysm, the decline was relatively slow; normal temperatures (below 37.2°C) were restored between 5 and 7 hr after the onset of the chill.

In one of the nine patients only, the relationship between chill, rigor and sweating and the course of rise and fall of temperature was atypical. The TNF levels were also highly atypical. The reasons for this aberrance are unknown, and this patient is excluded from the following data presentation and discussion.

The dynamics of the rise and fall in TNF levels closely followed the dynamics of temperature change (Fig. 1), the rise and fall in TNF appearing to precede that of temperature by 30–60 min.

Although the dynamic relationship between TNF levels and temperature was remarkably close in eight patients, the absolute levels of TNF were highly variable among the patients, ranging at peak from ≈100 pg/ml to 3000 pg/ml (Figs. 2 and 3; see also Fig. 1). To determine a correlation between TNF levels and temperature in all patients, it was therefore necessary to normalize both TNF levels and temperature in individual patients. In each patient, peak TNF levels and peak temperatures were set at 100 normalized TNF or temperature units and all other measurements of temperature and TNF in the patient were expressed in normalized units. A general correlation between TNF and temperature was ascertained by combining the normalized data from all patients. In Fig. 4*A*, normalized temperature is plotted against the normalized TNF value that corresponds to the same point in time during the paroxysm. A Spearmann correlation coefficient (r) of 0.7 was found.

However, as noted above (Fig. 1), the rise and fall of TNF appeared to precede those of temperature by 30–60 min. We therefore plotted the normalized temperatures against normalized TNF values at time points 15, 30, 45, 60, and 75 min (lag time) prior to the time of each temperature point. The TNF values at these time points were extrapolated from graphs such as Fig. 1 that were constructed for each patient. Correlations between normalized temperature and normal-

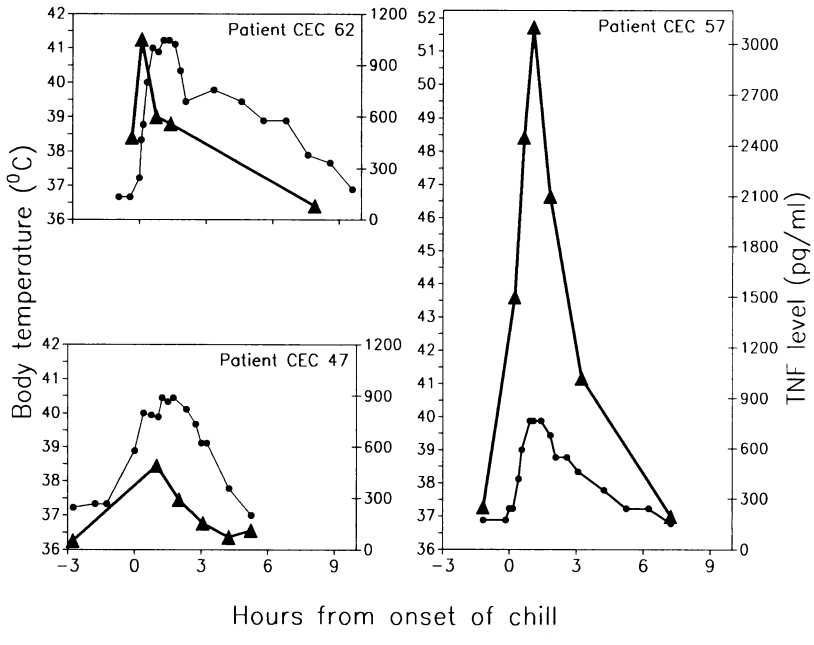

FIG. 2. Body temperature (●) and plasma TNF level (▲) during the course of a single paroxysm in three of the *P. vivax* patients studied. A single scale has been used for each of the parameters in all three patients to show differing absolute plasma TNF levels in different patients.

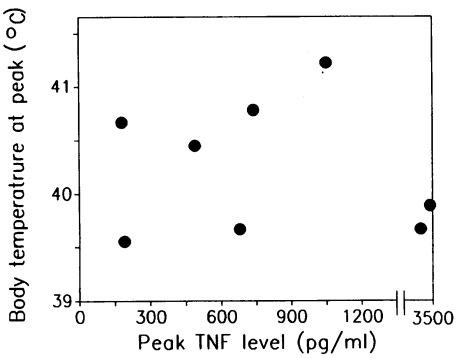

FIG. 3. Correlation between peak TNF levels and the body temperature at peak, during the course of a single paroxysm in eight *P. vivax* patients.

ized TNF values of all patients were ascertained for each of the above lag times. Intervals of 30 or 45 min (Fig. 4*B*) gave the best correlations ($r = 0.78$, Spearmann rank correlation). Other lag times gave poorer correlations, suggesting that the changes in TNF levels generally precede those of temperature by 30–45 min.

Serum TNF levels have been measured by others during *P. falciparum* infections (5, 6, 10). Because it is generally not ethically justified to follow patients through a paroxysm in *P. falciparum* malaria, because of the potential life-threatening nature of infection with this parasite, TNF measurements have been taken, in effect, at random following admission of a patient to hospital. Therefore, to make a comparison of TNF levels during *P. vivax* infections with those previously measured in *P. falciparum* infections, we made a series of TNF measurements in serum samples taken at random from 25 other *P. vivax*-infected patients, following their admission to the hospital. The mean serum TNF levels in such patients were compared with those reported by Kwiatkowski *et al.* (6)

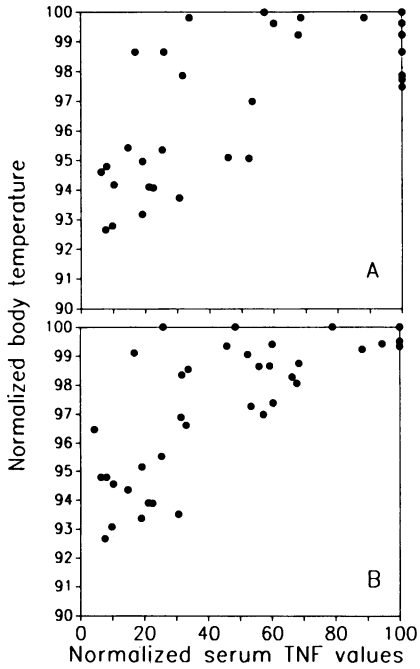

FIG. 4. Correlation in eight *P. vivax* patients between normalized body temperature and normalized TNF level, which corresponds in *A* to the same time as, and in *B* to the level 45 min prior to, the time for each temperature point. Values were normalized by setting the peak TNF and peak body temperature values in each patient at 100 normalized units and expressing all other measurements of temperature and TNF in that patient as normalized units.

for *P. falciparum*-infected patients experiencing mild symptoms, severe malaria (cerebral malaria not leading to death), and cerebral malaria leading to death (Table 1). Random serum TNF levels in *P. vivax* infections were higher than those in mild *P. falciparum* and as high as those in severe cases. Only in lethal *P. falciparum* infections were random TNF levels higher than in *P. vivax* infections. Even these levels, however, were much lower than the peak serum TNF levels achieved in the *P. vivax* patients followed throughout paroxysm in the present study.

DISCUSSION

We have studied the relationship between immunoreactive plasma TNF levels and fever and other symptoms during paroxysm in nine patients with primary infections of *P. vivax* malaria. In all but one of these patients the rise and fall in temperature followed closely the rise and fall in TNF levels, but the changes in temperature lagged behind the changes in TNF levels by 30–45 min. The duration of the fever and elevated TNF serum levels varied between 5 and 9 hr in different patients. Other symptoms—chills, rigor, and sweating—were relatively short episodes during and immediately following, in sequential order, the early sharp rise in the temperature and TNF levels.

These data suggest that the rise and fall in TNF levels may be closely associated with the mechanism controlling temperature changes during *P. vivax* malaria paroxysm. However, the lag of up to 1 hr between changes in TNF levels and changes in temperature may have been simply due to the fact that we measured the oral temperature, changes in which might in fact have lagged behind changes in the core body temperature. Alternatively, this lag may indicate that other intermediaries may intervene between TNF and the direct cause of body temperature change. It is also possible that TNF is not by itself involved in the mechanisms of temperature change but is produced simultaneously with other factors that are. Other cytokines that may be involved in the pathogenesis of malarial fever include interleukin 6, which has been implicated as a mediator of lipopolysaccharide-induced fever in rats (11). As we have reported (4), in 70 patients with *P. vivax* malaria, serum levels of interleukin 6 were elevated during paroxysms; interleukin 1 levels, on the other hand, did not show a rise. Similarly, in African children with *P. falciparum* malaria, no increase in serum interleukin 1α was found (6).

Although changes in body temperature closely paralleled changes in serum TNF in individual patients, the levels of TNF itself varied greatly between different patients in whom the temperature rise was similar. Temperature change is, however, a likely response to the biologically active component of TNF, which in these patients might have been different from the immunoreactive TNF levels that were

Table 1. Comparison of plasma TNF levels in *P. vivax* and *P. falciparum* patients

Disease and severity (no. of patients)	Time of plasma collection	Plasma TNF,* pg/ml
P. vivax (8)	Peak paroxysm	746.14 (264.6–2104.1)
(25)	Random	54.98 (17.38–173.8)
P. falciparum†	Random	
Mild (178)		24 (20–29)
Severe (cerebral) (82)		51 (36–72)
Fatal (28)		269 (170–431)

*Geometric mean (and 95% confidence intervals) of TNF levels measured in both studies by immunoassays.
†Data for *P. falciparum* were derived from ref. 6.

measured. These differences may reflect differences in the ability of patients to neutralize and/or inactivate TNF *in vivo*. It is likely that the production of soluble TNF receptors was increased in these patients, and this deserves further study. However, high levels of soluble TNF receptors are unlikely to have interfered with our immunoradiometric assay for TNF; it has been demonstrated that the addition of recombinant soluble TNF receptors, even at concentrations exceeding 1000 ng/ml, did not have any effect on the recovery of TNF in this particular assay (12).

While we have previously reported on an association between serum TNF, other as yet unidentified serum factors, and "disease" symptoms during *P. vivax* malaria (4), we know of no previous reports on TNF in relation to fever in *P. vivax* malaria. Several studies have also been made on TNF and disease in *P. falciparum* malaria. For ethical reasons it is difficult to follow patients through a complete paroxysm of *P. falciparum* malaria, because of the life-threatening nature of the disease. However, studies have been done on the correlation between TNF levels and prognosis for pathology and mortality in *P. falciparum* patients. In two studies out of three (5, 6, 8) there has been a clear correlation between severity of disease (mild disease, nonfatal cerebral malaria, and fatal cerebral malaria) and TNF levels. However, in a pilot clinical trial using an anti-TNF antibody in *P. falciparum* patients, no effect was found upon morbidity or mortality, although a significant reduction in fever was found in the treated patients (Kwiatkowski, D., Molyneux, M. E., Pointaire, P., Curtis, N., Klein, N., Smit, M., Allan, R., Stephens, S., G.E.G., Holloway, P., Brewster, D. R. & Greenwood, B. M., unpublished results). Thus, as in *P. vivax* malaria, the strongest direct association of TNF in *P. falciparum* malaria has been with fever rather than with other aspects of pathology.

It has been postulated that TNF in *P. falciparum* may be involved in the mechanism of cerebral malaria (5, 6). In this context, we note that the mean levels of TNF measured at random in *P. vivax* patients are as high as or higher than those in patients with mild *P. falciparum* infections or nonlethal *P. falciparum* infections with cerebral complications. Indeed, the peak concentrations of serum TNF measured in *P. vivax* infections far exceed those measured in any *P. falciparum* infection, 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. While TNF may indeed be involved in these effects of *P. falciparum* infection, it would have to be in conjunction with other properties of this parasite not associated with *P. vivax*. Such properties could involve the sequestration of parasite-infected erythrocytes in the vascular circulation, including that of the brain, characteristic of *P. falciparum* (13) but not *P. vivax*. It could also be that the short pulse of TNF due to *P. vivax* paroxysm is insufficient to induce severe manifestations or threaten life. The duration for which high serum TNF levels were sustained in the *P. falciparum* infections is not known but may well have exceeded that in the *P. vivax* infections, as high temperatures (shown here to closely parallel high TNF levels) are typically maintained for much longer periods (>24 hr) in *P. falciparum* infections. The possibility must also be considered that the severe and life-threatening aspects of *P. falciparum* infections may not be related at all to TNF. In trials of the effects of an anti-TNF monoclonal antibody on the severity of *P. falciparum* infections, no difference in mortality was found between treated and control groups. A significant difference was found, however, in the height of fever in the two groups, with the patients receiving anti-TNF antibody having temperatures reduced compared with those in control groups (Kwiatkowski, D., Molyneux, M. E., Pointaire, P., Curtis, N., Klein, N., Smit, M., Allan, R., Stephens, S., G.E.G., Holloway, P., Brewster, D. R. & Greenwood, B. M., unpublished results).

In conclusion, these and our present observations indicate that there is a close correlation between the rise and fall of temperature during human malarial infections and the rise and fall in serum TNF levels. From our present observations, the changes in TNF levels generally precede those in temperature by 30–45 min. This suggests that other intermediaries than TNF may be involved in the elevation or reduction of temperature in the infected host. It is not proven by our studies, however, that TNF is directly involved in the temperature changes during a malarial paroxysm. It is possible that this cytokine is merely produced in parallel with other factors which themselves mediate malarial fever, but that TNF itself plays no direct part. This question might be resolved by an intervention study in *P. vivax* patients in which either (*i*) the effects of TNF are neutralized by the administration of an anti-TNF antibody prior to the onset of a paroxysm or (*ii*) TNF levels are kept low by the use of TNF synthesis inhibitors.

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