Quarterly Journal of Medicine, 1993, 86: 91–98

Anti-TNF therapy inhibits fever in cerebral malaria

D. KWIATKOWSKI, M.E. MOLYNEUX, S. STEPHENS, N. CURTIS, N. KLEIN, P. POINTAIRE, M. SMIT, R. ALLAN, D.R. BREWSTER, G.E. GRAU and B.M. GREENWOOD

From the Department of Paediatrics, Oxford University, UK; Liverpool School of Tropical Medicine, UK; Celltech Ltd, Slough, UK; Department of Pathology, University of Geneva, Switzerland; Royal Victoria Hospital, Banjul, The Gambia; and MRC Laboratories, Fajara, The Gambia

Received 26 October 1992; Accepted 14 December 1992

Summary

The clinical effects of a murine monoclonal antibody (CB0006) directed against tumour necrosis factor were investigated in an open study of 41 Gambian children receiving otherwise conventional therapy for cerebral malaria. Ten children received a single i.v. dose of CB0006 at 0.1 mg/kg, 10 received 1 mg/kg, 10 received 5 mg/kg, and 11 were randomly selected as controls. CB0006 rapidly formed complexes with tumour necrosis factor, which were cleared from the circulation over several days. This was associated with a dose-dependent increase in total plasma tumour necrosis factor levels and a dose-dependent reduction of fever, implying that CB0006 inhibits tumour necrosis factor by retaining it in the circulation and reducing its availability to tissue receptors. Parasite clearance rates were not impaired. The fatality rate (29% overall) was similar in CB0006-treated patients and controls, but evaluation of possible effects on mortality requires a much larger blinded study. These data show that tumour necrosis factor is involved in the pathogenesis of malaria fever, and are the first direct evidence that inhibition of a specific endogenous pyrogen can attenuate fever in man.

Introduction

Malaria fever is one of the commonest causes of morbidity in the tropics. A minority of episodes have fatal complications (primarily cerebral malaria or profound anaemia due to *Plasmodium falciparum*) which are reckoned to cause at least one million childhood deaths annually in Africa alone.[1] The precise mechanism of malaria fever and the critical factors in the pathogenesis of severe malaria are poorly understood.

There is now considerable interest in the role of tumour necrosis factor in both malaria fever and cerebral malaria. Three observations suggest that tumour necrosis factor is involved in malaria fever: it is an endogenous pyrogen;[2] schizont rupture stimulates its release by human monocytes *in vitro*;[3] and paroxysms of fever are immediately preceded by a sharp rise in circulating tumour necrosis factor levels in patients with vivax malaria.[4] Febrile temperatures inhibit parasite growth[5] and tumour necrosis factor has other anti-parasitic effects,[6] so in normal circumstances this response is probably protective. An excessive response, however, may

Presented in part at the Third International Workshop on Cytokines, Stresa, Italy, Nov 1991. Abstract in Cytokine 1991; 3:497.

Address correspondence to Dr D. Kwiatkowski, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU

be deleterious. The clinical evidence for this is an association between circulating tumour necrosis factor levels and disease severity in falciparum malaria,[7–9] and the observation that high tumour necrosis factor levels correlate with fatality in childhood cerebral malaria better than other laboratory markers such as parasitaemia or blood glucose levels.[9] Experimental data suggest several reasons why excessive tumour necrosis factor production might promote cerebral malaria,[6] apart from its general pathological effects as seen in severe bacterial sepsis.[10] Specifically, it may exacerbate the sequestration of parasites in cerebral blood vessels by upregulating adhesion molecules, such as ICAM-1, that mediate the binding of *Plasmodium falciparum* to vascular endothelium.[11] High local levels of tumour necrosis factor produced in areas of dense sequestration are likely to stimulate endothelial release of nitric oxide, which would tend to raise intracranial pressure and might also disturb neuronal conduction in the surrounding brain.[12] Human cerebral malaria lacks a close experimental model, but it has certain pathological features in common with a lethal cerebral syndrome caused by *P. berghei* anka infection in CBA mice, which can be completely aborted by antibodies to tumour necrosis factor.[13]

At the Royal Victoria Hospital in Banjul, The Gambia, cerebral malaria accounts for 20% of pediatric admissions, and cases with an admission coma score of $\leqslant 2$ have a fatality rate of 30%. There is an urgent need for better forms of treatment for cerebral malaria, and anti-tumour necrosis factor therapy clearly merits investigation. Various forms of such therapy are being developed by major pharmaceutical companies but so far there is little published information about its clinical use. Therefore, in preparation for a double-blind mortality study (requiring a sample size of several hundred), we investigated the safety and biological efficacy of a monoclonal anti-tumour necrosis factor antibody in an open study of 41 children with cerebral malaria. Our results prove that tumour necrosis factor is involved in the pathogenesis of malaria fever.

Patients and Methods

Monoclonal antibody

CB0006 (Celltech, UK) is an IgG_1 murine monoclonal antibody that neutralizes both recombinant and natural human tumour necrosis factor *in vitro*. Toxicology studies indicate a safe profile, and no significant adverse effects were observed when doses of up to 10 mg/kg were administered intravenously to adults with septic shock.[14]

Patient selection and clinical management

The study was approved by the Gambian Government/MRC Joint Ethical Committee. Informed consent was obtained from the parents or guardians of the patients concerned. It was carried out in the children's ward of the Royal Victoria Hospital, Banjul, The Gambia during a 6-week period in October and November 1990, when all comatose children over 1 year old were examined on admission by a member of the study team. A child was provisionally admitted to the study if he had a Blantyre coma score[15] $\leqslant 2$ on arrival, asexual *P. falciparum* parasitaemia, and no clinically evident cause of coma other than malaria. Lumbar puncture was performed, unless clinically contraindicated, to exclude meningitis. Blood glucose was measured by test strip (BM-Test-Glycemie 1-44) using a Reflolux S meter (Boehringer Mannheim) and any child with a level of less than 2.2 mM/l immediately received 1 ml/kg of 50% glucose i.v. Convulsions were treated initially with intramuscular paraldehyde (0.15 ml/kg). All patients received i.m. chloroquine sulphate 3.5 mg base/kg on admission and 6 hourly (or 2.5 mg/kg orally 6 hourly when conscious) to a total dose of 25 mg/kg, in accordance with standard policy in The Gambia at that time.

Children were reassessed 1 h after arrival and those whose coma score remained $\leqslant 2$ despite correction of hypoglycaemia were formally assigned a study number. Treatment was determined by a random sequence contained in sealed envelopes. Those randomized to the control group received standard treatment only: no placebo was given. Those randomized to receive CB0006 were treated in ascending doses: the first 10 received 0.1 mg/kg, the next 10 received 1 mg/kg, and the final 10 received 5 mg/kg. CB0006 was given by a single i.v. injection over 15 min.

Temperature, pulse, blood pressure, coma score, and general clinical condition were recorded every 15 min for the first hour, hourly for 4 h, 3-hourly for 24 h, and subsequently every 6 h. Rectal temperature was monitored using an ET401 meter (Libra Medical). Temperatures above 39°C were treated with tepid sponging and fanning, and above 40°C with 10 mg/kg of rectal paracetemol.

Persistent convulsions were treated in the following sequence: (1) rectal diazepam 0.2 mg/kg with a second dose if necessary; (2) i.m. paraldehyde 0.1 mg/kg; (3) i.m. phenobarbitone 10 mg/kg. The standard fluid regimen for the duration of coma was 4% dextrose with 0.18% saline at 80 ml/kg/day, modified according to individual needs. Blood glucose was monitored by test strip 3- to 6-hourly; levels of less than 2.2 mM/l were treated with additional doses of intravenous 50% glucose.

Laboratory investigations

Small sequential blood samples were obtained from an indwelling venous cannula during the first 72 h after treatment. Levels of CB0006, immunoreactive TNF, TNF complexed with CB0006, and human anti-mouse antibodies were measured on plasma (EDTA 1 mg/ml; aprotinin 0.5 TIU/ml) which was separated immediately. TNF bioactivity was measured on serum separated 1 h after collection. All samples were stored at −20°C and underwent only one freeze-thaw cycle prior to assay, which was performed without knowledge of the treatment received. Additional fingerprick samples were taken 12-hourly for parasitaemia until two consecutive blood films were negative. All survivors were examined, and a further blood sample taken, 2–3 weeks after commencing treatment.

Parasites were counted in relation to 1000 erythrocytes (on Giemsa stained thin films) or 200 leucocytes (on Field-stained thick films). Red and white cell counts were taken into account when calculating parasite density.

CB0006 levels were measured by a competitive enzyme-linked immunosorbent assay. Microtitre wells were coated with recombinant human tumour necrosis factor: 50 μl sample plus 150 μl of CB0006 conjugated to horseradish peroxidase at 0.125 μg/ml were incubated for 2 h. After washing, tetramethylbenzidine was added and colour development measured at 630 nm. CB0006 levels were estimated from a standard curve included on each plate.

Immunoreactive tumour necrosis factor levels were measured by immunoradiometric assay (Medgenix, Fleurus, Belgium) as previously described.[7] Samples were incubated overnight with ^{125}I-labelled monoclonal antibody to recombinant human tumour necrosis factor, in tubes coated with a mixture of three different monoclonal antibodies to recombinant human tumour necrosis factor. Tubes were then washed and counted, and tumour necrosis factor levels determined with reference to a standard curve included in each assay.

Bioactive tumour necrosis factor in serum was measured as previously described[16] in terms of its cytotoxic effect on the fibrosarcoma cell line WEHI 164 clone 13 (obtained from Dr. T. Espevik, Trondheim, Norway and subsequently sub-cloned) using recombinant human tumour necrosis factor standards.

Tumour necrosis factor complexed with CB0006 was measured by enzyme-linked immunosorbent assay. Microtitre wells were coated with goat anti-rabbit immunoglobulin followed by polyclonal rabbit anti-tumour necrosis factor antibody. The sample, diluted 1:2 in PBS with 1% BSA, was incubated for 1 h. After washing, horseradish peroxidase-conjugated goat anti-mouse immunoglobulin Fc-region was added for 30 min. After another washing tetramethylbenzidine was added and colour development read at 630 nm. The amount of complexed tumour necrosis factor was estimated from a standard curve containing variable amounts of tumour necrosis factor with an excess (2 μg/ml) of CB0006.

Human anti-mouse antibodies were measured by enzyme-linked immunosorbent assay. Samples were incubated for 1 h in microtiter wells coated with CB0006. After washing, CB0006 conjugated to HRP was added for 1 h. After another washing tetramethylbenzidine was added and colour development measured at 630 nm. Results were expressed relative to a negative control serum.

IL-6 levels were measured by an enzyme-linked immunosorbent assay (Medgenix, Fleurus, Belgium). Samples were incubated in microtitre wells coated with a monoclonal antibody to human recombinant IL-6. Wells were washed and incubated with a different anti-IL-6 monoclonal antibody conjugated to horseradish peroxidase. After further washing, tetramethylbenzidine substrate was added. The reaction was stopped with H_2SO_4 and colour development at 450 nm was compared with that of hrIL-6 standards.

Results

Admission data

Twenty-five boys and 16 girls entered the study. Ten were given 0.1 mg/kg of CB0006, 10 had 1 mg/kg, and 10 had 5 mg/kg; 11 were controls. The four groups were similar in average age (respectively: 3.8 year, range 1.1–8.2; 3.9 year, 1.4–7.0; 4.4 year, 1.8–8.0; 4.5 year, range 1.8–8.0) and in weight (13.5 kg, range 8–21; 12.4 kg, 8–20; 14.3 kg, 9–22; 14.6 kg, 9–25). There was no significant difference between the groups in the admission haematocrit, the number requiring blood transfusion, or the number with plasma glucose below 2.2 mM/l on admission (the respective values were 24%, 3, 2; 25%, 2, 3; 25%, 2, 2; 31%, 1, 3).

Fever

Rectal temperature declined sharply in all groups during the first 12 h but subsequently rose again in the control group, whereas it continued to fall in CB0006-treated patients (Fig. 1). The time to become afebrile, defined as the time until rectal temperature fell to ≤37.6°C for at least 24 h, was significantly less in the 5 mg/kg group (median 15 h, range 9–96 h) than in controls (60 h, 18–192 h;

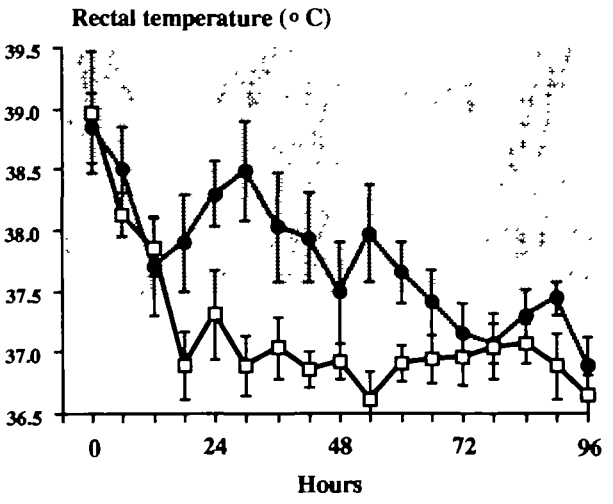

Figure 1. Mean temperature in patients treated with 5 mg/kg of CB0006 (□, $n=7$) and controls (●, $n=8$). Bars represent standard error. To show an uninterrupted sequence, these data are for survivors only; however the results are similar when fatal cases are included in the analysis.

$p=0.02$ by 2-tailed Mann-Whitney test). Intermediate values were observed in the 1 mg/kg group (26 h, 12–150 h) and 0.1 mg/kg group (57 h, 30–96 h).

To analyse this in more detail, the area under the fever curve was calculated for each survivor and expressed as a fever index (Table 1). In the first hour after treatment this value was similar in all four groups, but over the 72 h period following treatment it was significantly lower in the 5 mg/kg treated group ($p=0.04$, 2-tailed Mann-Whitney test) than controls. The 0.1 and 1 mg/kg groups had intermediate values, and non-parametric analysis of all survivors by Jonckeere's W-test was consistent with a dose-dependent effect of CB0006 on fever ($p<0.007$).

A similar effect was observed when fatal cases were included in the analysis, by calculating a fever index up to the time of death (Table 1). However because of the small number of deaths, over half of which occurred within 24 h, it was not possible to determine whether CB0006 had a specific effect on fever in those who died.

Parasite clearance

A history of chloroquine use in the previous 24 h was obtained from 53% of the CB0006-treated group and 55% of controls. Mean parasitaemia on admission was similar in all treatment groups, as was the initial rate of parasite clearance (Fig. 2). At 72 h parasitaemia was lower in the 1 mg/kg and 5 mg/kg groups than in the control group, but this difference was not significant, allowing for multiple comparisons. Although all patients initially responded to chloroquine, at 14–21 days 12/29 (41%) of survivors still had detectable parasitaemia. This did not significantly differ between treated and untreated groups (four of eight controls, five of eight of the 0.1 mg/kg group, one of six of the 1 mg/kg group, and two of eight of the 5 mg/kg group). In most cases the parasitaemia was $<1000/\mu l$ and the child was well. Clinical recrudescences of fever with parasite densities of over $75\,000/\mu l$ were observed in three patients at 8, 14 and 22 days after commencing treatment: all had received the lowest dose of CB0006. These recrudescences responded promptly to treatment with Fansidar. These data indicate that chloroquine is becoming less effective in The Gambia.[17]

Table 1 Fever index

CB0006 dose (mg/kg)	0–1 h (survivors)	0–72 h (survivors)	0–72 h (all cases)
0	38.8 (0.3)	38.0 (0.3)	38.6 (0.4)
0.1	39.0 (0.4)	37.7 (0.2)	37.9 (0.2)
1	38.8 (0.4)	37.4 (0.2)	37.8 (0.2)
5	38.8 (0.4)	37.1 (0.2)	37.5 (0.3)

Mean fever index (with standard error) representing the average fever over the period of observation, calculated as the area under the temperature curve divided by the duration of observation. For fatal cases the fever index represents the average fever up to the time of death.

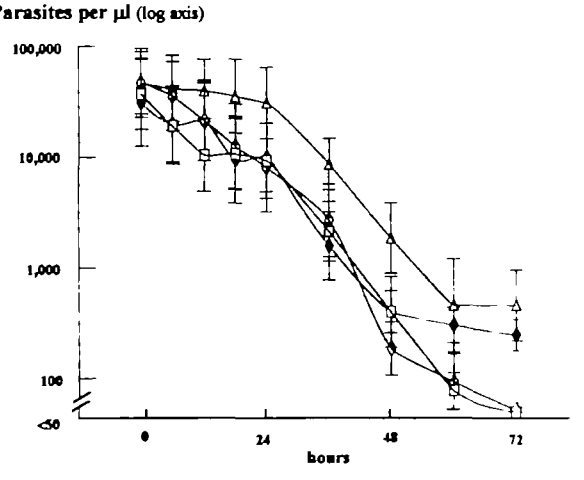

Figure 2. Geometric mean parasitemia in patients given CB0006 at 5 mg/kg (□), 1 mg/kg (◇), or 0.1 mg/kg (△) compared with controls (◆). Bars represent standard error.

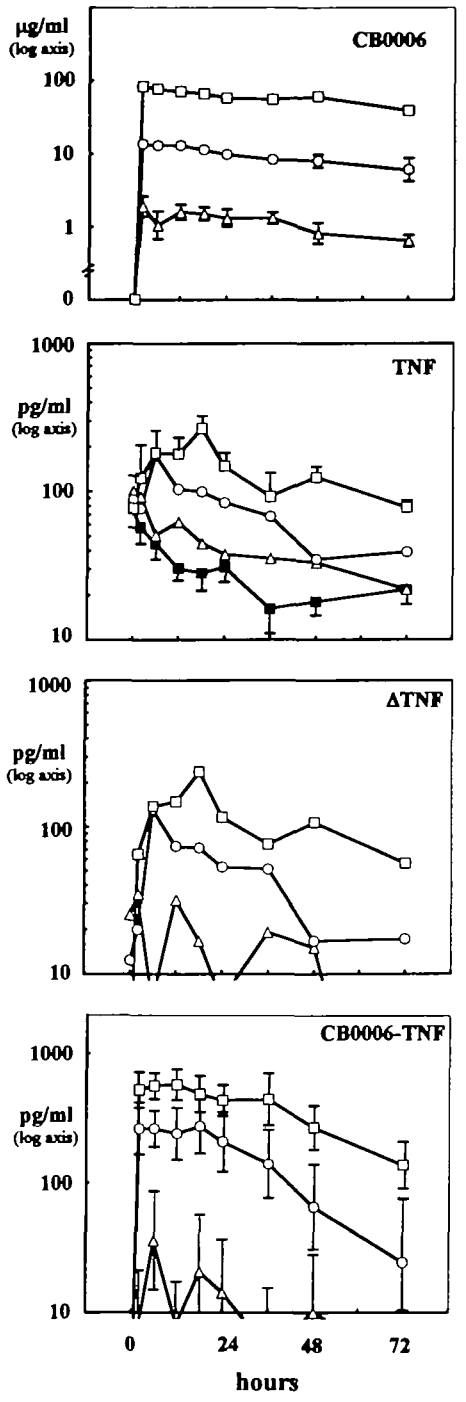

Figure 3. (a) Plasma CB0006 levels (geometric mean and standard error: where error bars are not visible they fall within the printed symbol). (b) Plasma TNF levels (geometric mean: standard error shown for 5 mg/kg group and controls). (c) ΔTNF, which represents the excess of immunoreactive TNF levels in CB0006-treated patients compared to the controls, calculated as described in the results section and shown here as a geometric mean. (d) TNF complexed with CB0006 (geometric mean and standard error).

□ patients given 5 mg/kg CB0006; ○, 1 mg/kg; △, 0.1 mg/kg; ●, controls. To show an uninterrupted sequence, these data are for survivors only; however the results are similar when fatal cases are included in the analysis.

Levels of CB0006, tumour necrosis factor, and tumour necrosis factor bound to CB0006

Two hours after treatment, geometric mean CB0006 levels were 1.9 μg/ml (range 0.2–4.5) in the 0.1 mg/kg group, 13.5 μg/ml (6.9–20.4) in the 1 mg/kg group, and 83.2 μg/ml (49.3–119.4) in the 5 mg/kg group (Fig. 3a). Overall, the level at 48 h was 51% of that at 2 h. These data are consistent with a single compartment model of distribution, since CB0006 distributed in a plasma volume of 57 ml/kg (for a child of 13.7 kg with a haematocrit of 25%) should theoretically achieve levels of 1.7 μg/ml at 0.1 mg/kg, 17 μg/ml at 1 mg/kg, and 88 μg/ml at a dose of 5 mg/kg—figures that are close to the observed values at 2 h.

Circulating tumour necrosis factor levels on admission, measured by immunoradiometric assay, were similar in CB0006-treated patients and controls. Thereafter levels declined steadily in controls but rose in CB0006-treated patients (Fig. 3b). The difference between the 5 mg/kg group and controls was significant at each of the sample times from 6 to 72 h ($p < 0.005$ by 2-tailed t-test on log-transformed data) and intermediate levels were observed in the 1 mg/kg and 0.1 mg/kg groups. Tumour necrosis factor bioactivity was below the detection limit of the assay (150 pg/ml of recombinant human tumour necrosis factor) in all samples except one. This was an admission sample with an unusually high level of immunoreactive tumour necrosis factor (2059 pg/ml) and bioactivity equivalent to 270 pg/ml of recombinant human tumour necrosis factor.

CB0006–tumour necrosis factor complexes appeared in plasma within 2 h of treatment and remained for several days (Fig. 3d). Four patients gave a false-positive result on admission and were therefore excluded from this analysis. The amount of tumour necrosis factor bound to CB0006 was related to dose: the geometric mean at 6 h was 562 pg/ml in the 5 mg/kg group, 263 pg/ml in the 1 mg/kg group, and 36 pg/ml in the 0.1 mg/kg group.

We examined whether tumour necrosis factor retained in the circulation by CB0006 might explain the rise in circulating levels observed after treatment. To do this, we calculated the difference between the observed levels of tumour necrosis factor and the mean level in the control group at each time point. This difference (ΔTNF, shown in Fig. 3c) correlated well with the amount of tumour necrosis factor bound to CB0006 at 2 h ($r = 0.55$, $p < 0.01$), 6 h ($r = 0.60$, $p < 0.001$) and 12 h ($r = 0.61$, $p < 0.001$).

IL-6 levels

We observed no difference in IL-6 levels between controls and any of the three treatment groups. In survivors, plasma IL-6 levels fell rapidly over the first 6 h. In controls, the geometric mean level on admission was 197 pg/ml (95% C.I. 93–414); at 2 h, 90 pg/ml (43–190); at 6 h, 57 pg/ml (33–99); at 24 h, 64 pg/ml (19–215); and at 72 h, 52 pg/ml (32–85). In patients treated with CB0006, the mean admission level was 194 pg/ml (106–356); at 2 h, 113 pg/ml (65–196); at 6 h, 64 pg/ml (45–92); at 24 h, 66 pg/ml (48–90); and at 72 h, 36 pg/ml (29–44). In some of the fatal cases IL-6 levels rose after admission: this was observed in both treated patients and controls. The sample size was too small for detailed analysis of the effect of CB0006 on IL-6 levels in those who died.

Human anti-mouse antibody

The human anti-mouse antibody (HAMA) response to treatment was expressed as the ratio (HAMA at 3 weeks)/(pre-treatment HAMA) for each patient. A significant response was noted in all CB0006-treated patients (mean ratio 22.1, SD 14.3) and in none of the control group (mean ratio 0.8, SD 0.3). The level of response was unrelated to CB0006 dose: in the 0.1 mg/kg group it was 27.7 (SD 12.6); in the 1 mg/kg group, 14.5 (13.0); and in the 5 mg/kg group, 23.0 (16.1).

Clinical outcome

Overall 12 children (29%) died, five (12%) had major neurological sequelae, and the remainder were discharged between 4 and 14 days after commencement of treatment. Mortality and sequelae rates did not significantly differ between the four treatment groups. Three of the control group died, compared to two of those receiving 0.1 mg/kg group, four of the 1 mg/kg group, and three of the 5 mg/kg group. Major neurological sequelae occurred in two, one, one, and one, respectively. The median time to death was 18 h (range 6–28) in controls and 24 h (range 12–64) in those who received CB0006. Among survivors, the median time to achieve a coma score of 5 was 21 h (range 6–288) in controls and 24 h (1–228) in the treated group. All survivors were back to their normal activities within 1 month of admission, with the exception of two controls (both hemiplegic) and 1 child who received CB0006 (who had generalized weakness and irritability). No adverse reactions to CB0006 therapy were noted.

Discussion

This study was undertaken in order to establish whether monoclonal anti-tumour necrosis factor antibodies can safely and effectively inhibit the biological activity of tumour necrosis factor in children with cerebral malaria. We found that children treated with CB0006 in addition to conventional antimalarials had a dose-dependent reduction in fever, with no adverse effects.

An important conclusion is that tumour necrosis factor is involved in the pathogenesis of malaria fever. This has implications for fever in general, which is believed to be mediated by a family of cytokines (known as endogenous pyrogens) that stimulate prostaglandin E synthesis in the thermoregulatory centre of the hypothalamus: these include TNF, interleukin-1, and interleukin-6.[18] This concept of a common fever pathway is founded on a number of classical experimental investigations, but in naturally occurring fever it has proved difficult to demonstrate that any specific endogenous pyrogen has a critical role[19]. Our data provide strong support for the view that natural fever is mediated through specific endogenous pyrogens, and show for the first time that inhibition of an endogenous pyrogen can attenuate fever in man. The antipyretic properties of anti-tumour necrosis factor therapy in cerebral malaria do not seem to be due to an indirect effect on IL-6 production, as circulating IL-6 levels fell at a similar rate in CB0006-treated patients and controls.

This result is also of practical significance in relation to malaria vaccine development. Older children in endemic regions become able to tolerate high levels of parasitemia without fever or other symptoms, and there is growing interest in the possibility that a similar state of clinical tolerance could be induced by an 'anti-disease' vaccine.[20] Current attempts to develop such a vaccine are based on the assumption that tumour necrosis factor is a critical mediator of malaria fever, and these data provide the first direct evidence that this is so.

A surprising finding was that circulating tumour necrosis factor levels (measured by immunoassay) rose after treatment with CB0006, though the clinical effects of tumour necrosis factor (manifest by fever) were evidently inhibited. This apparent paradox can be simply explained as follows. Tumour necrosis factor is generated in the circulation in malaria, particularly after schizont rupture.[3,4] Free tumour necrosis factor is rapidly cleared by receptor binding, with a plasma half-life in the order of minutes.[21] Our data show that CB0006 rapidly formed complexes with tumour necrosis factor, and that these complexes remained in the circulation for several days. The amount of tumour necrosis factor bound to CB0006 was found to correspond closely to the observed rise in total circulating

levels. These observations indicate that CB0006 acts to retain tumour necrosis factor within the circulation, rather than enhancing its clearance as we had supposed. Our results imply that tumour necrosis factor retained in the circulation is less biologically active, at least in respect of pyrogenicity, because of reduced availability to tissue receptors.

These findings shed light on the general question of clinical tumour necrosis factor measurement by immunoassay and its relationship to bioactivity. Several groups have reported high plasma levels of tumour necrosis factor in falciparum malaria,[7–9] but it has not been clear how much of this circulating cytokine is bioactive. The immunoradiometric assay used in this study detects both bioactive and biologically inactive tumour necrosis factor that is complexed with binding proteins such as its soluble receptor,[22] plasma levels of which are elevated in malaria (M.E. Molyneux *et al.*, unpublished data). Our patient with the highest immunoreactive tumour necrosis factor level on admission (2059 pg/ml) had bioactivity equivalent to 270 pg/ml of recombinant human tumour necrosis factor, and in all other patients bioactivity was below the detection limit of the assay (150 pg/ml of recombinant human tumour necrosis factor), suggesting that most of the circulating tumour necrosis factor in cerebral malaria patients is complexed to inhibitory binding proteins. This does not mean that overall activity is low, as the low circulating levels of free tumour necrosis factor may simply reflect its rapid exit to the tissues. The clinical effects of tumour necrosis factor are more likely to be determined by its turnover than its blood levels. The prompt rise in plasma tumour necrosis factor levels that we have observed in response to CB0006 injection (which effectively enlarges the pool of tumour necrosis factor binding proteins) suggests that the turnover of this cytokine in cerebral malaria is high.

As expected, patients treated with CB0006 developed antibodies to murine immunoglobulin. This was not associated with any obvious side-effects, and is probably of little clinical importance in this context because recurrent cerebral malaria is uncommon so repeated treatments with monoclonal antibodies would rarely, if ever, be indicated. However this result does have methodological implications as these antibodies can cause false positive results in immunoassay methods that use murine IgG. It is therefore worth pointing out that the measurements of tumour necrosis factor CB0006 reported in this paper were made during the first 3 days of treatment, before the rise in human anti-mouse antibodies was detected.

The primary purpose of this study was to explore the possibility of using anti-tumour necrosis factor therapy in the treatment of cerebral malaria. A potential drawback is that tumour necrosis factor (and fever) has anti-parasitic as well as pathological properties, and inhibition of this response could theoretically cause a delay in parasite clearance. In practice, this is likely to depend on the efficacy of the antimalarials given together with anti-tumour necrosis factor therapy. We observed similar rates of parasite clearance in CB0006-treated patients and controls during the first 48 h: intriguingly, at 72 h parasitaemia was lower in the 1 mg/kg and 5 mg/kg CB0006-treated groups than in controls. This result is encouraging but may have been confounded by the considerable variability in chloroquine sensitivity of *P. falciparum* in The Gambia, and a larger sample size is needed to address this question conclusively.

The critical question is whether anti-tumour necrosis factor therapy can reduce mortality in cerebral malaria. In this hospital, the overall case fatality rate of cerebral malaria is around 20%, and for those with a Blantyre coma score of 2 or less on admission it is 30%. To detect a 33% reduction in fatality in the latter group with a power of 80% at $p < 0.05$ requires a double-blind placebo-controlled trial in about 600 patients. The present study is too small to draw any meaningful conclusions about mortality but it has shown that monoclonal anti-tumour necrosis factor antibodies can reduce the bioactivity of this cytokine in cerebral malaria patients without obvious side-effects. On the basis of these results, a much larger trial is now in progress.

Acknowledgements

We are grateful to the staff of the Royal Victoria Hospital for their help with this study. We thank Simon Jennings and Paul Holloway for advice, and David Warrell, Richard Moxon and Janice Giffen for support. This study received funding from the Tropical Disease Research programme of WHO and the MRC. DK is an MRC Senior Research Fellow.

References

1. Sturchler D. How much malaria is there worldwide? *Parasitol Today* 1989; 5:39–40.
2. Dinarello CA, Cannon JG, Wolff SM, *et al* Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin-1. *J Exp Med* 1986; **163**:1433–50.
3. Kwiatkowski D, Cannon JG, Manogue KR, Cerami A, Dinarello CA, Greenwood BM. Tumour necrosis factor production in falciparum malaria and its association with schizont rupture *Clin Exp Immunol* 1989; **77** 361–6
4. Karunaweera ND, Grau GE, Gamage P, Carter R, Mendis KN. Dynamics of fever and serum TNF levels are closely

associated during clinical paroxysms in *Plasmodium vivax* malaria. *Proc Natl Acad Sci USA* 1992; **89**:3200–3.

5. Kwiatkowski D. Febrile temperatures can synchronise the growth of *Plasmodium falciparum in vitro*. *J Exp Med* 1989, **169**:357–61.
6 Clark IA. Cell-mediated immunity in protection and pathology of malaria. *Parasitol Today* 1987; **3**:300–5.
7. Grau GE, Taylor TE, Molyneux ME, *et al*. Tumor necrosis factor and disease severity in children with falciparum malaria. *N Engl J Med* 1989; **320**:1586–91
8. Kern P, Hemmer CJ, van Damme J, Gruss H-J, Dietrich M Elevated tumor necrosis factor alpha and interleukin-6 serum levels as markers for complicated *Plasmodium falciparum* malaria. *Am J Med* 1989, **57**:139–43
9. Kwiatkowski D, Hill AVS, Sambou I, *et al*. TNF concentration in fatal cerebral, non-fatal cerebral, and uncomplicated *Plasmodium falciparum* malaria. *Lancet* 1990; **336**:1201–4.
10. Tracey KJ, Vlassara H, Cerami A. Cachectin/tumour necrosis factor. *Lancet* 1989; **i**:1122–6.
11 Berendt AR, Simmons DL, Tansey J, Newbold CI, Marsk K. Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for *Plasmodium falciparum*. *Nature* 1989; **341** 57–9.
12 Clark IA, Rockett KA, Cowden WB. Proposed link between cytokines, nitric oxide, and human cerebral malaria. *Parasitol Today* 1991; **7**:205–7.
13 Grau GE, Fajardo LF, Piguet P-F, Allet B, Lambert P-H, Vassali P Tumor necrosis factor (Cachectin) as an essential mediator in murine cerebral malaria. *Science* 1987; **237**:1210–2.
14. Exley AR, Cohen J, Buurman W, *et al* Monoclonal antibody to TNF in severe septic shock. *Lancet* 1990; **335**:1275–7.
15 Molyneux ME, Taylor TE, Wirima JJ, Borgstein A. Clinical features and prognostic indicators in paediatric cerebral malaria: a study of 131 comatose Malawian children. *QJ Med* 1989; **71** 441–59.
16 Espevik T, Nissen-Meyer J. A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes *J Immunol Methods* 1986, **95**:99–105
17. White NJ, Krishna S, Waller D, Craddock C, Kwiatkowski D, Brewster D. Open comparison of intramuscular chloroquine and quinine in children with severe chloroquine-sensitive falciparum malaria. *Lancet* 1989; **ii** 1313–6
18. Dinarello CA. Interleukins, tumor necrosis factors (cachectin), and interferons as endogenous pyrogens and mediators of fever. *Lymphokines* 1987, **14**:1–31.
19. Kluger MJ. Fever: role of pyrogens and cryogens. *Physiol Rev* 1991; **71**:93–127.
20 Playfair JHL, Taverne J, Bate CAW, de Souza JB. The malaria vaccine: anti-parasite or anti-disease? *Immunol Today* 1990; **11** 25–7.
21 Beutler B, Milsark IW, Cerami A. Cachectin/tumor necrosis factor: production, distribution and metabolic fate in vivo. *J Immunol* 1985; **135**:3972–7.
22. Engelberts I, Stephens S, Francot GJM, van der Linden CJ, Buurman WA. Evidence for different effects of soluble TNF receptors on various TNF measurements in human biological fluids *Lancet* 1991; **338**:515–6