The Effect of a Monoclonal Antibody to Tumor Necrosis Factor on Survival from Childhood Cerebral Malaria

Michaël Boele van Hensbroek, Ayo Palmer, Emeka Onyiorah, Gisela Schneider, Shabbar Jaffar, Grainne Dolan, Hannah Memming, Joost Frenkel, Godwin Enwere, Steve Bennett, Dominic Kwiatkowski, and Brian Greenwood*

Tumor necrosis factor (TNF) is thought to play a key role in the pathogenesis of cerebral malaria. A double-blind, placebo-controlled trial of an anti-TNF monoclonal antibody (B-C7) comprised 610 Gambian children with cerebral malaria, with mortality and residual neurologic sequelae as primary study end points. Sixty (19.9%) of 302 children who received B-C7 died compared with 64 (20.8%) of 308 children who received placebo (adjusted odds ratio [OR], 0.90; 95% confidence interval [CI], 0.57–1.42). Residual neurologic sequelae were detected in 15 (6.8%) of 221 survivors from the B-C7 group and in 5 (2.2%) of 225 survivors of the placebo group (adjusted OR, 3.35; 95% CI, 1.08–10.4). The monoclonal antibody used in this study did not improve survival in cerebral malaria and was associated with a significant increase in neurologic sequelae. A possible explanation of the latter observation is that the antibody acts to retain TNF within the circulation and thereby prolongs its effects on vascular endothelium.

Cerebral malaria in children has a case fatality rate of 10%–30% despite effective antimalarial treatment [1, 2]. Deaths in cases of cerebral malaria occur frequently in the first hours after admission, before antimalarial therapy has become effective. Recently, it has been shown that a fast-acting antimalarial agent, artemether, did not change this pattern, nor did it improve outcome [3]. Thus, there is a need to develop new forms of therapy for cerebral malaria. These should have antidisease properties and a fast onset of action and be given in conjunction with conventional antimalarial treatment.

Recent studies strongly suggest that tumor necrosis factor (TNF) plays a key role in the pathogenesis of cerebral malaria. In clinical studies, an association has been found between plasma TNF levels and disease severity and outcome [4, 5], and it has been shown that children homozygous for the TNF2 allele, which has been associated with high TNF production, are more likely to develop cerebral malaria and die as a consequence [6]. Two in vitro sets of observations provide explanations of why overproduction of TNF may be important in the pathogenesis of cerebral malaria.

First, TNF can up-regulate expression of intracellular adhesion molecule-1, which in turn may facilitate the sequestration of parasite-infected erythrocytes in the cerebral blood vessels, a process that is thought to be central to the pathogenesis of cerebral malaria [7].

Second, TNF stimulates nitric oxide production, which in turn may raise intracranial pressure and disturb neuronal conductivity within the brain [8]. Furthermore, experimental murine models of malaria, though not directly analogous with the human disease, have indicated that some aspects of the cerebral pathology can be prevented by administration of antibodies to TNF [9].

Taken together, these observations suggest that it might be possible to improve the outcome of cerebral malaria by treatment with drugs that interfere with the release of TNF or with its action, although it is possible that by the time children present with coma, it may be too late to do so. To investigate whether this is the case, we undertook a trial of treatment of cerebral malaria with a monoclonal antibody (Mab) to TNF.

As a first step, the safety and biologic activity of a mouse anti-TNF Mab (CB0006; Celltech, Slough, UK) were tested in a open study on 41 Gambian children with cerebral malaria. CB0006 was safe and reduced fever in a dose-dependent fashion [2]. Because production of CB0006 was discontinued, a safety study of a second mouse anti-TNF Mab (B-C7; Diaclon, Besançon, France) was undertaken in 38 Gambian children with cerebral malaria. No significant side effects were recorded, and a trend was found toward a reduction in mortality and neurologic sequelae in children treated with B-C7 compared with a placebo group (Kwiatkowski D, unpublished data).

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Informed consent to participate in the study was obtained from the parents or guardians of the study children. The study was approved by the Gambian Government/Medical Research Council Laboratories Ethical Committee.

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Reprints or correspondence: Dr. M. Boele van Hensbroek, Dept. of Tropical Medicine F4, Academical Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam, Netherlands.

* Present affiliation: Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, United Kingdom.
On the basis of these encouraging preliminary findings, we undertook a large double-blind, placebo-controlled trial of B-C7 therapy in cerebral malaria with mortality and residual neurologic sequelae as the major study end points.

Methods

The study was started in 1992 on the pediatric ward of the Royal Victoria Hospital (RVH) and was expanded in 1993 to include the Sibanor Health Centre (SHC). Data on admission characteristics, mortality, neurologic sequelae, and coma recovery time were recorded at both centers. Sequential observations on parasite and fever clearance were collected only at RVH. All unconscious children ages 1–9 years who presented at either hospital were eligible if they fulfilled the following selection criteria: Blantyre coma score ≤2 [10]; asexual forms of Plasmodium falciparum present in a thick blood film; and informed consent of parent or guardian. Patients with concomitant disease at the time of admission and patients with coma who responded to intravenous glucose treatment or who recovered consciousness within 1 h after a convolution were excluded.

Study design. An anti-TNF MAb (B-C7) and placebo were compared in a double-blind trial. This formed part of a larger cerebral malaria intervention study, in which a new antimalarial agent, artemether, was also evaluated. A 2 × 2 factorial design was used to allow B-C7 to be compared with placebo while artemether and quinine were compared in the same groups of patients. The statistical principles of the factorial design are well established [11, 12]; the results of the comparison between the antimalarials will be published elsewhere.

The primary end points of the study were mortality in hospital and residual neurologic sequelae. Secondary end points were parasite and fever clearance rates, coma recovery time, and neurologic sequelae at discharge and 1 month after admission. On the basis of our previous experience of a 25%–30% mortality rate in this group of patients and data from the pilot studies, we aimed to randomize sufficient children to be able to show a 30% reduction in mortality. A study with 580 children would have had an 80% probability of detecting this difference at the 5% significance level.

The treatment code for each child was kept in a sealed envelope, which was opened after the admission procedure was completed and consent had been obtained. Each envelope contained a card indicating a letter (A–K) corresponding to 1 of 10 treatment vials (5 with B-C7 and 5 with placebo) and the antimalarial treatment (artemether or quinine) to be given. To ensure that disease severity was well matched between the treatment groups, randomization was stratified by admission coma score (0, 1, or 2) and by study center (RVH or SHC) and balanced over time in blocks of 10 patients across both anti-TNF and antimalarial therapy.

Anti-TNF antibody. B-C7 is a murine IgG1κ MAb that blocks the cytotoxic activity of natural and recombinant human TNF in the murine cell line L929. The antibody is safe and biologically active in patients with acute graft-versus-host disease [13]. Vials of B-C7 contained the MAb, human serum albumin (0.1%), PBS, and water for injection. The placebo vials lacked the antibody. The placebo and B-C7 vials appeared identical. The antibody (in a dose of 5 mg/kg) or placebo was given as a single intravenous infusion over a 15-min period. Infusion was started immediately after the first antimalarial treatment was given, normally within 1 h of admission.

Patient care and follow-up. On admission, a clinical history was obtained and physical examination done. A blood sample was collected for malaria diagnosis, blood glucose measurement (Haemo-glucotest; Boehringer Mannheim, Mannheim, Germany), and determination of packed cell volume (PCV) and for blood culture, hematology, and biochemistry. Lumbar puncture was done, unless clinically contraindicated, to exclude meningitis. Hypoglycemia, defined as a blood glucose level of <2.2 mmol/L, was treated with 1 mL/kg 50% glucose. Convulsions were treated with diazepam (rectal, 0.5 mg/kg; intravenous, 0.3 mg/kg) or paraldehyde (intramuscular, 0.1 mL/kg). Blood (15 mL/kg) was transfused if the PCV was <15%. Suspected secondary infections were treated with chloramphenicol (25 mg/kg every 6 h) for a minimum of 5 days.

Patients allocated to the artemether group received intramuscular injections of Paludrine (Rhône-Poulenc, Antony Cedex, France) into the anterior thigh for 4 consecutive days, in an initial dose of 3.2 mg/kg followed by daily doses of 1.6 mg/kg. Patients allocated to the quinine group received intramuscular injections of quinine dihydrochloride (Rotemmedica, Hamburg, Germany, obtained via the International Dispensary Organization [IDO], Amsterdam) into the anterior thigh for 5 consecutive days, in an initial dose of 20 mg/kg followed by 10 mg/kg every 12 h. Once a patient was able to swallow, intramuscular injections were replaced by oral quinine sulfate (Pharmamed, Valletta, Malta, obtained via IDO, Amsterdam). In the second and third study years, oral pyrimethamine-sulfadoxine (in a dose as close as possible to 1.25/25 mg/kg) was added to the antimalarial treatment regime, in order to reduce the high recrudescence rate seen at 1-month follow-up in the first year.

Vital signs were recorded every 4 h for the first 24 h and then every 6 h until discharge. Blood glucose measurements were repeated after 4 and 12 h and on clinical indication. Blood films for parasite density were obtained every 12 h, and the PCV was measured daily.

Immediately before discharge, each patient underwent physical examination with neurologic assessment. A child was diagnosed as having neurologic sequelae if he or she had at least one of the following abnormalities: paresis, ataxia, spasticity, general floppiness, hearing defects, visual field defects, aphasia, behavior abnormalities, or developmental regression. Patients were seen again 1 month after admission for detailed neurologic assessment. This included a questionnaire on the child’s behavior and performance and a detailed examination by a clinical investigator.

Subjects who had neurologic sequelae at 1 month were reviewed by the same clinical investigator at their 6-month (the following dry season) follow-up. Those without evident sequelae at 1 month were visited at their 6-month follow-up by a field worker; if there was any doubt concerning the child’s health or performance, he or she was referred to the clinical investigator for further evaluation. Of the survivors, 95.3% were reviewed at 1 month and 92.0% at 6 months after admission. The 2 treatment groups were similar in the proportion of cases lost to follow-up (at 1 month, 13/242 [5.4%] in the B-C7 group and 10/244 [4.1%] in the placebo group; at 6 months, 20/241 [8.3%] in the B-C7 group and 19/244 [7.8%] in the placebo group), most frequently due to migration out of Gambia or incorrect addresses.

Statistical methods. The protocol specified that two initial procedures should be completed before a full analysis of the data was
undertaken. First, because of the factorial design, it was necessary to examine whether randomization to artemether versus quinine might have affected the difference in results between B-C7 and placebo. Second, a multiple logistic regression model was used to identify admission variables that affected clinical outcome. In the case of death, these were temperature, pulse rate, hypoglycemia, coma score, and study center; in the case of neurologic sequelae, they were the duration of coma, coma score, and hypoglycemia. These factors were considered to be potentially confounding, and we adjusted for them and for antimalarial treatment in a multiple logistic regression model when the effect of B-C7 on outcome was analyzed.

Discrete data were analyzed by the χ² test or Fisher’s exact test, with stratified analysis by the Mantel-Haenszel test and multivariate analysis by unconditional logistic regression. Continuous secondary end points that were normally distributed were analyzed by the t test, after application of Bartlett’s test for homogeneity of variance; those that were not normally distributed were analyzed by the Wilcoxon test. Survival times were compared using Kaplan-Meier plots and the log-rank test, with multivariate analysis by Cox regression. Different multivariate models were compared by using the likelihood ratio test.

The conduct of the study was monitored by the Tropical Disease Research Program of the World Health Organization, and the major outcome measures were reviewed annually by an independent monitoring committee. A locked data base and detailed analytical plan were submitted to the monitoring committee before the blinded randomization codes were broken at the end of the study.

Results

Patient Characteristics

In total, 624 children who fulfilled the study criteria were randomized to receive either B-C7 or placebo, 388 at RVH and 236 at SHe. Nine children did not receive their study drugs, either because they died before treatment could be given (3 children) or because of unavailability of the study drug (6 children). Five children died during infusion. All 624 randomized children are included in an intention-to-treat analysis, but only the 610 children who actually received their full treatment. The clinical characteristics of the 2 groups were similar on admission (table 1).

Mortality and Neurologic Sequelae

Survival rates and the incidences of neurologic sequelae are given in table 2 and figure 1.

Mortality. The mortality rates (death in hospital) were 19.9% of children in the B-C7 group and 20.8% of those in the placebo group (P = .9). The adjusted odds ratio (OR) for death for children in the B-C7 group was 0.90 (95% confidence interval [CI], 0.57–1.42). An intention-to-treat analysis led to the inclusion of 14 additional children, 8 in the B-C7 group and 6 in the placebo group. The mortality rate was slightly higher in both groups (21.0% and 22.0% in the B-C7 and placebo groups, respectively) than in the previous analysis, but the adjusted OR was similar (0.88; 95% CI, 0.73–1.78; P = .6). As illustrated in figure 1, the survival patterns of children who died were similar in the 2 groups, as were the median times to death (13.5 h with an interquartile range [IQR] of 5–27 h and 14 h with an IQR of 7–25 h for B-C7 and placebo, respectively; P = .7). The effect of B-C7 was not influenced by the duration of coma before treatment was started. One hundred one children had a history of coma of ≤2 h before treatment was given. Seven deaths occurred among the 47 children who received B-C7 and 6 among the 54 children who received placebo. One child in the B-C7 group who was well by the duration of coma before treatment was given. Seven deaths occurred among the 47 children who received B-C7 and 6 among the 54 children who received placebo. One child in the B-C7 group who was well at the time of discharge died at home 4 days later.

Neurologic sequelae. Significantly more children in the B-C7 group compared with the placebo group developed residual neurologic sequelae (6.8% vs. 2.2%, respectively; P = .04). The adjusted OR for sequelae for children in the B-C7 group was 3.35 (95% CI, 1.08–10.4; P = .02). By using an intention-to-treat analysis, the frequency of neurologic sequelae in the dry season was 6.7% in the B-C7 group and 2.2% in the placebo group, giving an adjusted OR of 3.28 (95% CI, 1.05–10.20; P = .03).

Factorial design. Because of the factorial design, we examined whether the differences in results between B-C7 and placebo were the same for children treated with artemether as for those who received quinine. Among B-C7 recipients, there were 24 deaths in the artemether group (n = 138), 32 in the quinine group (n = 142), and 4 in those excluded from the artemether/quinine analysis (n = 22). Among placebo recipients, there were 30 deaths in the artemether group (n = 145), 28 in the quinine group (n = 140), and 6 in those excluded from artemether/quinine analysis (n = 23). With regard to residual neurologic sequelae among survivors treated with B-C7, there were 5 cases in the artemether group (n = 105), 8 in the quinine group (n = 100), and 2 in those excluded from
Table 2. Survival and incidence of neurologic sequelae (NS) among children with cerebral malaria treated with the anti-TNF monoclonal antibody B-C7 \((n = 302)\) or placebo \((n = 308)\).

<table>
<thead>
<tr>
<th></th>
<th>B-C7, % (no.)</th>
<th>Placebo, % (no.)</th>
<th>Crude analysis OR (95% CI)</th>
<th>(P)</th>
<th>Adjusted analysis OR (95% CI)*</th>
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<tr>
<td><strong>Primary end points</strong></td>
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<tr>
<td>Death in hospital</td>
<td>19.9 (60/302)</td>
<td>20.8 (64/308)</td>
<td>0.95 (0.63–1.43)</td>
<td>.9</td>
<td>0.90 (0.57–1.42)</td>
<td>.6</td>
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<td>NS at 6 months†</td>
<td>6.8 (15/221)</td>
<td>2.2 (5/225)</td>
<td>3.20 (1.08–11.45)</td>
<td>.04</td>
<td>3.35 (1.08–10.4)</td>
<td>.02</td>
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<td><strong>Secondary end points</strong></td>
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<tr>
<td>NS on discharge</td>
<td>24.4 (59/242)</td>
<td>22.1 (54/244)</td>
<td>1.10 (0.80–1.52)</td>
<td>.6</td>
<td>1.15 (0.73–1.82)</td>
<td>.5</td>
</tr>
<tr>
<td>NS at 1 month†</td>
<td>11.0 (25/228)</td>
<td>6.4 (15/233)</td>
<td>1.80 (0.88–3.70)</td>
<td>.1</td>
<td>1.75 (0.86–3.56)</td>
<td>.1</td>
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NOTE. OR, odds ratio; CI, confidence interval.
* OR for deaths was adjusted for temperature, pulse rate, hypoglycemia, coma score, study center, and antimalarial treatment. OR for neurologic sequelae was adjusted for duration of coma prior to admission, coma score, and antimalarial treatment.
† Subjects at follow-up: 1 month, 95.3% (463/486); 6 months, 92.0% (446/485).

As part of the secondary end points of the study, incidences of neurologic sequelae were also recorded on discharge and at 1-month follow-up. Although the incidence at both time points was higher in the B-C7 group than in the placebo group, the differences did not reach the level of significance (table 2).
We found that treatment with B-C7, a murine MAb against human TNF, did not improve the clinical outcome of cerebral malaria in African children. The fatality rate was similar among children who received B-C7 and those who received placebo, and the incidence of neurologic sequelae was higher among those who received B-C7. We have considered a number of possible reasons to explain this pathophysiologic and clinically important negative finding.

One interpretation of our findings is that TNF is a marker of disease severity rather than a causal mediator of cerebral malaria. However, the wide body of evidence from in vivo as well as in vitro studies, summarized in the Introduction, argues against this suggestion and makes it necessary to consider other reasons why B-C7 was ineffective.

It is possible that B-C7 failed to neutralize TNF effectively. However, the inhibitory activity of the batch of MAb used in this study was confirmed by testing on L929 cells in vitro, and a previous study of B-C7 in graft-versus-host disease provides further evidence that the antibody is biologically effective, as a partial response was observed in three-quarters of the study patients [13]. One of the major clinical effects of TNF is fever and, in the present study, we observed a faster decline of fever in the children treated with B-C7 than in placebo recipients. This was apparent within 4 h of administration of antibody and continued for the duration of observation. The fever index for the first 24 h was lower in the B-C7 than in the placebo group (38.0 ± 0.62°C vs. 38.2 ± 0.72°C, respectively, \( P = .05 \)).

**Parasite clearance (figure 3).** There was a trend toward faster parasite clearance in the B-C7 group than in the placebo group (50% parasite clearance time, 13 ± 9.3 h and 18 ± 10.1 h, respectively; \( P = .2 \)). There was also a trend toward a lower recrudescence rate at 1-month follow-up in the B-C7 group than in the placebo group (9.0% and 13.9%, respectively, \( P = .2 \)).

**Coma recovery.** Eleven of the 486 children who lived and were discharged from hospital had not regained full consciousness, 7 in the B-C7 group and 4 in the placebo group. Among the 475 who did recover from coma, the median recovery time was 24 h (IQR, 13–48 h) in the B-C7 group and 22 h (IQR, 12–42 h) in the placebo group (\( P = .4 \)).

**Disease Complications and Side Effects**

The incidences of disease complications, defined as convulsions and hypoglycemic events after the start of treatment and the need for blood transfusion and the incidence of secondary infections were similar in the 2 groups. No significant side effects that could be attributed to the study drugs were recorded.

**Discussion**

We found that treatment with B-C7, a murine MAb against human TNF, did not improve the clinical outcome of cerebral malaria in African children. The fatality rate was similar among children who received B-C7 and those who received placebo, and the incidence of neurologic sequelae was higher among those who received B-C7. We have considered a number of possible reasons to explain this pathophysiologic and clinically important negative finding.

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was most pronounced among children who subsequently died (data not shown), who were the children identified previously as having the highest TNF levels, which persist until death [5]. This suppression of fever shows that B-C7 at the dosage used in our trial has neutralizing activity in vivo, but it is not known what degree of TNF inhibition is required to reverse the pathologic processes of cerebral malaria and whether the antibody used in our trial was able to achieve this inhibition.

An important consideration is the way in which B-C7 affects the distribution of TNF in the body. Normally TNF is cleared rapidly from the circulation, with a plasma half-life of 10 min, unless it is retained by soluble TNF receptors, which act as natural inhibitors. Anti-TNF antibodies can act as soluble TNF receptors by retaining TNF within the circulation [2]. We considered it to be likely that this antibody-bound TNF was effectively neutralized, since fever was suppressed in antibody-treated patients. However, evidence is emerging that TNF bound to an inhibitory protein may behave paradoxically. For example, although high levels of soluble TNF receptors are inhibitory, low levels can prolong the bioactivity of TNF, possibly by stabilizing the active trimeric conformation [14]. It has also been demonstrated that different chimeric MAbs that possess identical TNF-binding domains may be either beneficial or deleterious when administered to animals with septic shock, depending on their isotype, which in turn determines their biologic properties [15].

Such considerations may account for the paradoxical effects of B-C7 on neurologic sequelae and parasite clearance. It had been anticipated that anti-TNF treatment might adversely affect parasite clearance but that it would reduce sequelae, whereas the trends observed in this study were the reverse. In interpreting this result, it is important to recognize that the disease-causing parasites are entirely confined to the circulation. It is possible that, by retaining TNF within the circulation, B-C7 may have enhanced the action of TNF on endothelium and thus aggravated neurologic complications. Similarly, B-C7 treatment may have boosted TNF stimulation of phagocytes in the spleen and elsewhere in the circulation, thereby tending to accelerate parasite clearance. Although this interpretation of the data is speculative, it highlights the importance of understanding the pharmacodynamics of MAbs and other cytokine-blocking agents before use in clinical trials. A more straightforward therapeutic option may be to suppress TNF production with agents such as pentoxyphilline [16] or supplementary chloroquine [17].

Attempts to reduce the fatality rate of cerebral malaria in African children must confront the fact that half of the deaths occur within 12 h of hospital admission. It is possible that at the time anti-TNF therapy was given, irreversible pathophysiologic processes had been activated and that the administration of B-C7 was too late. Although there was no indication that the MAb was more effective in children with a short duration of coma, the fact that the study children were unconscious at admission indicates that mediators downstream from TNF had already been activated.

One of these downstream mediators is nitric oxide, which is produced by the endothelium and mononuclear cells in response to TNF and which is thought to diffuse into the surrounding brain and interfere with normal neuronal conduction [8]. Enhanced activity of the inducible form of nitric oxide synthase (the enzyme responsible for nitric oxide production) may persist, especially in mononuclear cells, for many hours after the initial stimulus has been removed; so, even if TNF is neutralized by an anti-TNF antibody, nitric oxide production could persist [18]. This could explain how an anti-TNF antibody might not improve the outcome of cerebral malaria, while still having an effect on malaria fever. A potential therapeutic strategy would be to inhibit the generation of nitric oxide, but this is still a controversial idea [19, 20], and the inhibitors available are currently too nonspecific [18] to allow clinical trials in the immediate future.

When weighing the various treatment options, it is important to recognize the lack of certain knowledge about the final cause(s) of death in cerebral malaria. One of the major reasons for conducting the present study was to determine the likely importance of TNF as a common stimulus for several of the different end-stage mechanisms that have been proposed.

Cerebral malaria remains a major cause of childhood mortality in Africa, and there is no evidence that faster-acting antimalarial drugs will reduce the scale of this problem [3]. There is an urgent need for more detailed understanding of the molecular pathogenesis of cerebral malaria, focused on the development of sustainable therapeutic strategies for this devastating condition.

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