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Malaria Mimicry with Tumor Necrosis Factor

Contrasts Between Species of Murine Malaria and Plasmodium falciparum

I. A. Clark,* J. D. MacMicking,† K. M. Gray,† K. A. Rockett,† and W. B. Cowden†

From the Division of Biochemistry and Molecular Biology, School of Life Sciences, and the John Curtin School of Medical Research,† Australian National University, Canberra, Australia*

Because* Plasmodium berghei *ANKA induces cerebral malaria and* P. vinckei *does not, the former has often been studied as a model for human falciparum malaria. It lacks, however, many of the systemic changes seen in the human disease. Because both of these murine models and the human disease have now been defined in terms of excess tumor necrosis factor (TNF) production, the authors have more closely examined the two murine models in this light to see which provides the better overall model for falciparum malaria. Administering TNF to malaria-infected mice did not cause cerebral symptoms nor breakdown of the blood–brain barrier, which is the hallmark of* P. berghei *ANKA cerebral malaria and is generally absent in human cerebral malaria. Tumor necrosis factor did, however, induce hypoglycemia and liver injury, pathology that is seen in terminal* P. vinckei *and falciparum malaria, but is absent in terminal* P. berghei *ANKA malaria. Plasma TNF and interleukin-6 (IL-6) also were found to be consistently higher in infections caused by* P. vinckei *than in those caused by* P. berghei *ANKA. The pathology of* P. vinckei *malaria is thus consistent with raised systemic levels of TNF and other cytokines, as is falciparum malaria. The authors therefore conclude that* P. vinckei *malaria, although lacking a cerebral component, is the better model for the human disease. (Am J Pathol 1992, 140:325–336)

The origin of the neurologic changes seen in falciparum malaria is uncertain, and undoubtedly complex. Mice infected with *Plasmodium berghei* ANKA develop distinctive cerebral lesions in which cerebral vessels are partly obstructed by monocytes adhering to vessel walls. There is associated vascular leakage, edema, and hemorrhage.[1,2] In marked contrast to what is observed in human cerebral malaria, parasitized red cells do not adhere to vascular walls, and the adhesion of monocytes to the endothelial cells of cerebral vessels, so prominent in the rodent infection, is absent in falciparum malaria. Likewise, *P. berghei* ANKA cerebral malaria lacks the systemic involvement, with manifestations such as hypoglycemia and liver damage, that is seen in falciparum malaria and *Plasmodium vinckei*–infected mice.[1,3] Nevertheless, in the absence of another more closely resembling the human disease, this rodent model has been analyzed with a view to understanding the neurologic changes seen in falciparum malaria.

Some years ago, our laboratory proposed that much of the pathology of malaria was mediated by cytokines, notably tumor necrosis factor (TNF).[4,5] Serum TNF proved to be increased in mice ill with *P. vinckei* infection[6] and in falciparum malaria[7] and, with the advent of recombinant TNF, we were able to duplicate certain changes common to both *P. vinckei* and *Plasmodium falciparum* by injecting this cytokine into mice early in the course of infection with any of several rodent malarias (reference 3, and unpublished data). As we noted, cerebral vascular lesions were conspicuously absent when TNF was administered to mice in these experiments.[3]

This concept of cytokine involvement in malarial pathology was expanded by Rest,[8] who proposed that TNF produced the lesions she had earlier described in the cerebral vessels of *P. berghei* ANKA-infected mice.[2] Grau et al.[9] subsequently reported that rabbit polyclonal antibody specific for mouse TNF prevented the onset of cerebral symptoms and pathology in these mice, and that the presence of cerebral pathology in rodent malaria correlated with increased serum levels of this cytokine. This group have also reported elevated serum levels of

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Address reprint requests to Dr. I. A. Clark, Division of Biochemistry and Molecular Biology, School of Life Sciences, Australian National University, Canberra, ACT 2601, Australia.

interleukin-6 (IL-6) in *P. berghei* ANKA infections[10] and refer to a study, yet to be reported in detail, in which they reproduced the lesions of *P. berghei* ANKA cerebral malaria by injecting TNF into normal mice.[11]

If, as has been proposed,[9,11] rodent cerebral malaria is a consequence of raised levels of circulating TNF, it should be possible to reproduce this syndrome by infusing TNF into mice. We therefore decided to expand our earlier studies, in which no cerebral pathology had been observed after only a single injection of r hu TNF had been given,[3] to include a range of doses and time courses of TNF administration. We also used r mu TNF in similar experiments. Because IL-6 is induced by TNF,[12,13] and its circulating levels rise in parallel with TNF after lipopolysaccharide (LPS) injection[13] and in sepsis patients,[14] we also compared measurements of plasma IL-6 in mice terminally ill with *P. vinckei* and mice with *P. berghei* ANKA cerebral malaria.

Materials and Methods

Mice and Parasites

CBA/Ca mice, male or female and 6 to 8 weeks of age, were used in all experiments. They had been bred and maintained under specific pathogen-free conditions at the Australian National University. The strain of *P. berghei* ANKA employed was obtained from G. E. Grau (University of Geneva, Switzerland), *P. vinckei vinckei* from F. E. G. Cox (Kings College, London, UK), and *Plasmodium chabaudi* and *Plasmodium berghei* K173 from stocks originally supplied by D. Walliker (Institute of Animal Genetics, Edinburgh, UK). Parasites were stored in liquid nitrogen and routinely passaged in mice, with recourse to frozen stocks when required. All infections were initiated with 10^6 parasitized red cells, injected intraperitoneally. Cerebral malaria developed in 80% to 90% of the *P. berghei* ANKA-infected mice, when parasitemias were 10% to 30%, on days 7 through 9 after infection. As expected, no other mice developed cerebral symptoms. Mice infected with *P. vinckei* and *P. berghei* K173 became systemically ill when parasitemias reached 70% to 80%, and *P. chabaudi* infections, which peaked at 30% to 35% parasitemia, induced no visible illness.

IL-6 Determination in Mouse Plasma

Cell Line and Reagents

The IL-6–dependent murine hybridoma cell line, B9, was obtained from L. A. Aarden (University of Amsterdam, Amsterdam, The Netherlands). The cells were grown in Dulbecco's minimum essential medium (DMEM) containing 10% fetal calf serum (FCS), 10 mmol/l (millimolar) HEPES, 2 mmol/l L-Glutamine, 2×10^{-5} mol/l (molar) 2-mercaptoethanol, penicillin (25 U/ml), and streptomycin (25 mg/ml), and supplemented with a source of IL-6 (33 μl of a human tonsil lymphokine preparation/10 ml culture medium, provided by H. Warren, Royal Canberra Hospital, Canberra, Australia). The cells were passaged at 5×10^4 cells/ml every 2 to 3 days.

Recombinant murine IL-6 (10U/50 μl, where 1 unit is the concentration of IL-6 that leads to half maximal [^{3}H]thymidine incorporation in the assay) was provided by R. Simpson (Ludwig Institute for Cancer Research, Melbourne, Australia), and [^{3}H]thymidine (specific activity approximately 45 Ci/mmol, code TRK.637) was purchased from Amersham Radiochemicals, Amersham, United Kingdom.

Bioassay for IL-6

Plasmas were obtained during the courses of *P. vinckei* and *P. berghei* ANKA infections by collecting blood into 1.5 ml microcentrifuge tubes (Eppendorf, Hamburg, Germany) containing 2.5 U heparin. Interleukin-6 then was measured using a [^{3}H]thymidine-incorporation assay modified from Aarden et al.[15] Duplicate test plasmas were serially double-diluted in 50 μl DMEM across 11 wells of a sterile 96 flat-bottom well plate (Nunclon Microwell, Nunc, Roskilde, Denmark). The B9 cells, washed once in IL-6–free culture medium, were added at 10^4 cells/well. The plates, with a final volume of 200 μl/well, were incubated at 37°C in 5% CO_2 for 44 to 48 hours. Each well then was pulsed with 1 μCi [^{3}H]thymidine, and the plates were harvested 5 hours later on a semiautomatic cell harvester (Cambridge Technologies, Cambridge, MA). Incorporated radioactivity was measured using a Beckman LS3801 beta-counter (Beckman Instruments Inc. Fullerton, CA). Sample IL-6 levels were calculated from the concurrently run IL-6 standard curve.

TNF Administration to Malaria-infected Mice

Cerebral Symptoms and Brain and Liver Histology

Recombinant hu TNF (Asahi; 2.2×10^3 Asahi units/μg protein) was given intravenously at 5 or 7.5 μg/day on 2 consecutive days, beginning when groups of 5 to 6 mice infected with *P. vinckei, P. chabaudi,* or *P. berghei* K173 had reached 15% to 25% parasitemia. Other mice, at the same stage of *P. chabaudi* infections, received 5, 5, and 10 μg r hu TNF intravenously on 3 consecutive days. Groups of *P. vinckei*–infected mice (15% to 25% parasit-

emia) received 10 μg r hu TNF intravenously once, for 2 days at 5 μg/day, or for 5 days at 2 μg/day, and *P. berghei* ANKA-infected mice were given r hu TNF for 7 days at either 0.5 or 1 μg/day, in each case by intraperitoneal osmotic pumps (model 2001; Alzet Corporation, Palo Alto, CA) inserted 1 day after infection. In addition, mice infected with *P. vinckei* (20% parasitemia) and *P. berghei* ANKA (3% to 4% parasitemia) received 2.5 μg r mu TNF intravenously on 2 consecutive days. Mice were killed with ether, and the brains and livers removed and fixed in 10% formol saline, processed to paraffin, cut at 5 μm and stained with hematoxylin and eosin.

Integrity of Blood–Brain Barrier

The integrity of the blood–brain barrier was initially examined by comparing the movement of Evan's blue dye in normal, uninfected mice and those terminally infected with *P. vinckei* or *P. berghei* ANKA. Evan's blue (C.I. 23860; Direct Blue 53, Sigma Chemical Co., St. Louis, MO) was diluted to 1% (wt/vol) in normal saline. Mice received a 100-μl intravenous injection and, 1 hour later, while anesthetized with avertin, had their vascular tree flushed with 4 ml saline introduced into the left cardiac ventricle. The brain then was carefully removed for photography.

The effect of r TNF on blood–brain barrier integrity then was examined. Five micrograms r hu TNF or 2.25 μg r mu TNF (5.75×10^4 U/μg protein, provided by Prof. W. Fiers) was administered intravenously on 2 consecutive days to *P. vinckei*–infected mice, carrying 35% to 40% parasitemias on day 1, and 1.88 or 3.75 μg r mu TNF was administered intravenously on 2 consecutive days to *P. berghei* ANKA-infected mice, carrying 3% to 4% parasitemias on day 1. The brains were prepared for examination as outlined above.

Determination of Blood Glucose Levels

Blood glucose levels were measured during the course of undisturbed *P. vinckei* and *P. berghei* ANKA infections, and in mice infected with each parasite at various times after they had received intravenous r mu TNF (details given in figure legends). Ten microliters of blood was collected from the tail into 40 μl 0.66 mol/l perchloric acid, centrifuged, and the supernatant stored at −20°C. Glucose determinations were performed using a Beckman Glucose Analyser 2 (Beckman).

Determination of Tumor Necrosis Factor Levels

Plasma TNF was measured during the course of undisturbed *P. vinckei* infections using an enzyme-linked immunosorbent assay (ELISA) modified from Sheenan et al.[16] Briefly, 96 flat-bottom well plates (Immulon II, Dynatech Labs, Alexandria, VA) were coated by overnight incubation at 4°C with a monoclonal antibody to TNF (0.2 μg/well of TN319:12, Celltech, Slough, United Kingdom). The plates then were washed six times with PBS containing 0.05% Tween 20, and again between each subsequent treatment. Samples were diluted 1:5 in R10 (RPMI 1640 supplemented with 10% FCS, 10 mmol/l penicillin and streptomycin, and 10 mmol/l HEPES) and duplicate 100 μl aliquots of each were added to the coated wells and incubated overnight at 4°C. A standard curve of r mu TNF (Genzyme, Boston, MA), starting at 25 ng/ml and followed by eight doubling dilutions in R10, was run concurrently. Rabbit anti-murine TNF (Genzyme) was diluted 1:750 in R10, 100 μl was added to each well, and the plates were incubated for 2 hours at 25°C. Goat anti-rabbit alkaline phosphatase (Silenus, Hawthorn, Victoria, Australia) was diluted 1:1000 in R10, 100 μl was added to each well, and the plates were incubated again for 2 hours at 25°C. Phosphatase substrate tablets (Sigma) were dissolved in substrate buffer at a concentration of 1 mg/ml, and 100 μl was added to each well for color development. The plates were then read on a Titertek Multiskan MC ELISA reader (Flow Laboratories, McLean, VA) at test wavelength 405 nm and reference wavelength 620 nm, and the TNF in the samples was calculated from the standard curve.

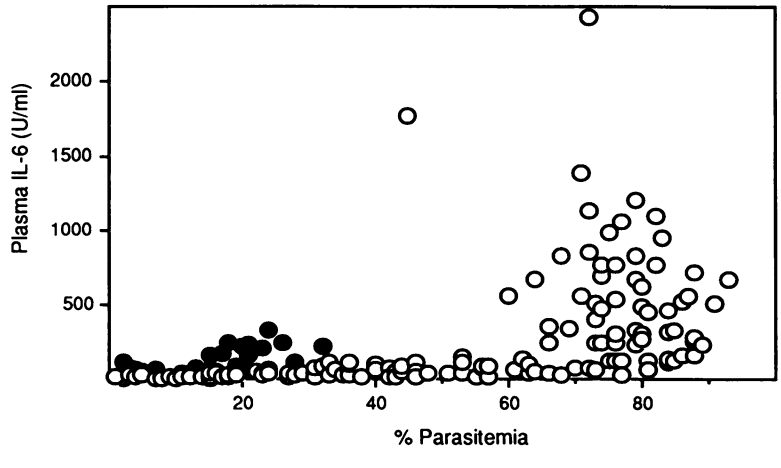

Figure 1. *Comparison of IL-6 levels during the course of* P. berghei *ANKA [●] and* P. vinckei *[○] infections. Symbols represent individual mice.*

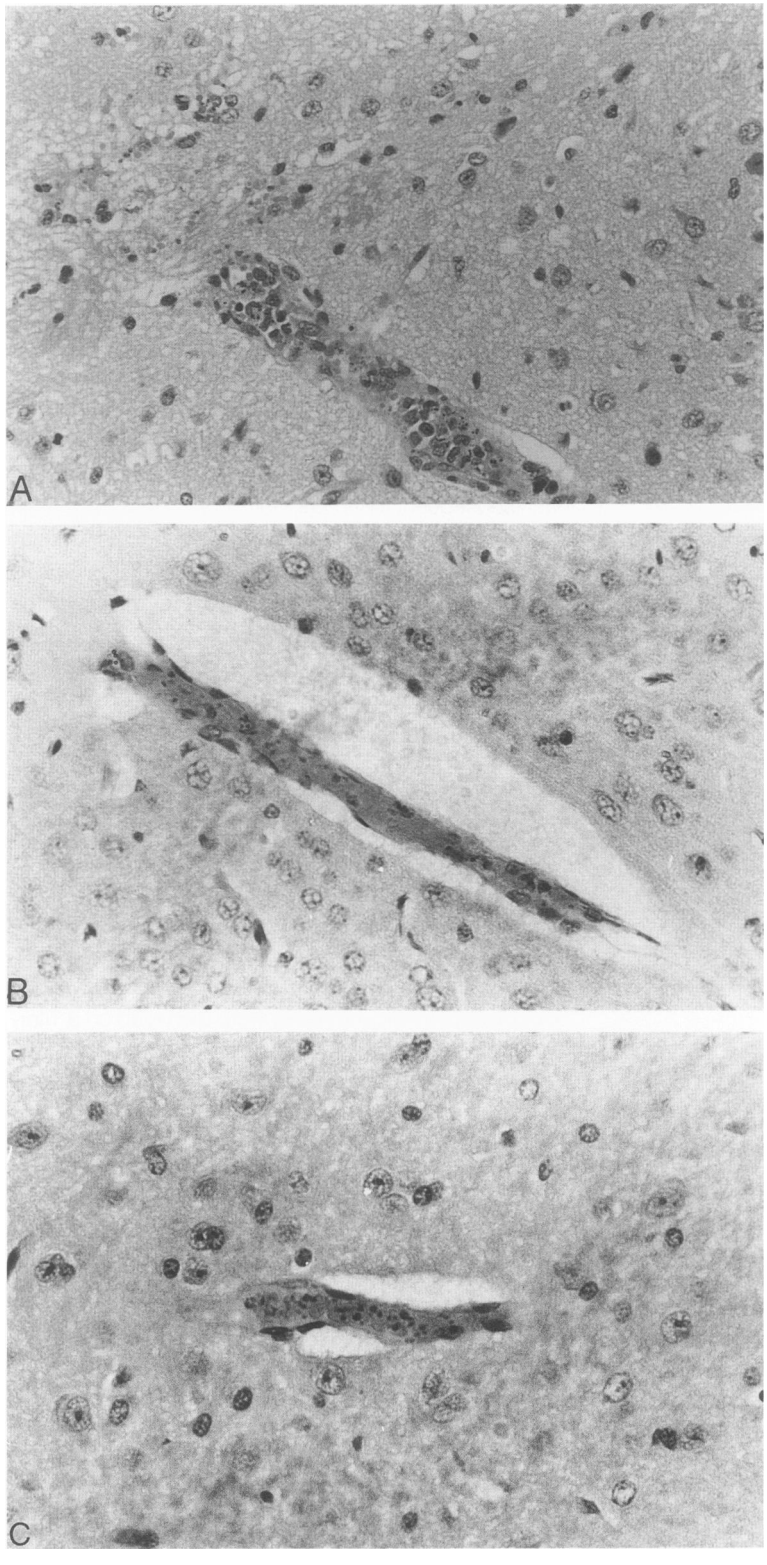

Figure 2. **A**: *Brain section from a* P. berghei *ANKA-infected mouse exhibiting cerebral symptoms. Monocyte adherence to endothelial cells present. (H&E, original magnification ×200).* **B**: *Brain sections from a low parasitemia* P. berghei *ANKA-infected mouse given 2.5 μg r mu TNF on 2 consecutive days and* **C**: *A mid parasitemia (51%)* P. vinckei-*infected mouse given 10 μg r hu TNF. Monocyte adherence to endothelial cells and haemorrhage absent (H&E, original magnification ×200).* **D**: *Brain section from a mouse infected with* P. chabaudi *given a total of 20 μg r hu TNF IV over 3 days. Monocyte adherence to endothelial cells present (H&E, original magnification ×200).*

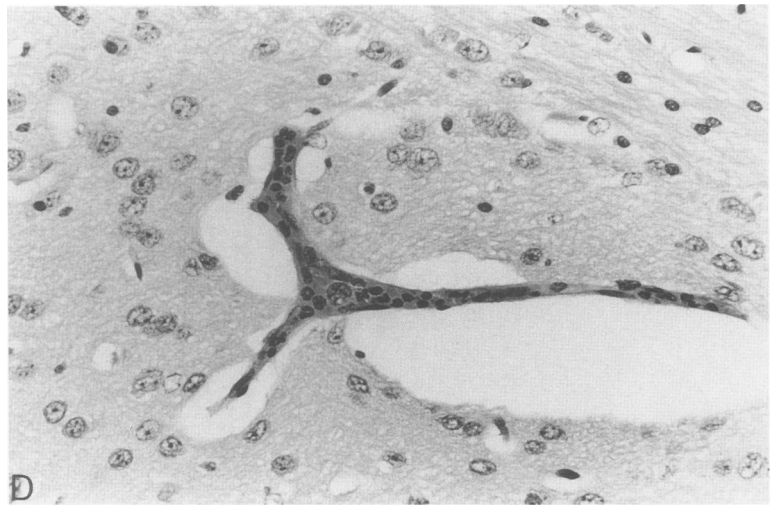

Figure 2. *(Continued).*

Statistical Analysis

Student's *t*-tests were applied to test the statistical significance of the data.

Results

Plasma IL-6 Levels in P. vinckei and P. berghei ANKA-infected Mice

Plasmodium berghei ANKA-infected mice were terminally ill within the 10% to 30% parasitemia range, whereas those infected with *P. vinckei* did not become ill until at least 75% of their red cells contained parasites. As shown in Figure 1, IL-6 levels rose considerably higher during the *P. vinckei* infections, commensurate with the higher TNF levels we have recently reported in these infections, compared with those induced by *P. berghei* ANKA.[17]

Outcome of TNF Administration to Malaria-infected Mice

Osmotic Pumps

Tumor necrosis factor was administered by 7-day osmotic pumps to 10 mice, five at the rate of 0.5 μg/day and the rest at 1 μg/day, with pumps being inserted 1 day after infection with 10^6 *P. berghei* ANKA. The higher but not the lower dose produced slight generalized illness, but neither dose precipitated cerebral malaria. This occurred equally and on the same day (day 9) in all three groups, the above two and a third in which the osmotic pumps contained saline alone. Earlier onset in the treated groups would have been consistent with plasma TNF level determining the onset of cerebral malaria in this model.

Intravenous Administration

To both lower the dose required[3] and to mimic the conditions of its production during the undisturbed course of the disease, intravenous TNF was given to mice during the early stages of malarial infection. *P. berghei* ANKA, *P. vinckei, P. chabaudi,* and *P. berghei* K173 infections were used.

Cerebral Symptoms and Brain Histology

All mice that received intravenous TNF showed degrees of the lethargy and piloerection characteristic of mice given *Escherichia coli* lipopolysaccharide (LPS) or r TNF, but in no case were the hind limb paralysis and other signs characteristic of *P. berghei* ANKA cerebral malaria, such as seizures, loss of balance, and forelimb paddling, observed.

On macroscopic examination of brains, no petechial hemorrhages, as routinely seen on the brain surface of mice ill with *P. berghei* ANKA cerebral malaria, were observed on the brains of mice that received TNF. Hemorrhages also were absent from histologic sections of brains from TNF-treated mice. Likewise the monocyte adherence to endothelial cells, which is characteristic of *P. berghei* ANKA cerebral malaria (Figure 2a), was generally absent after TNF administration (Figure 2b,c), the exceptions being in *P. berghei* K173-infected mice with high parasitemias, whether or not TNF had been administered (not shown), and the *P. chabaudi*-infected mice given a total of 20 μg r hu TNF over 3 days (Figure 2d). The adherence was not extensive and was present in limited areas of the brains only. In neither case was it

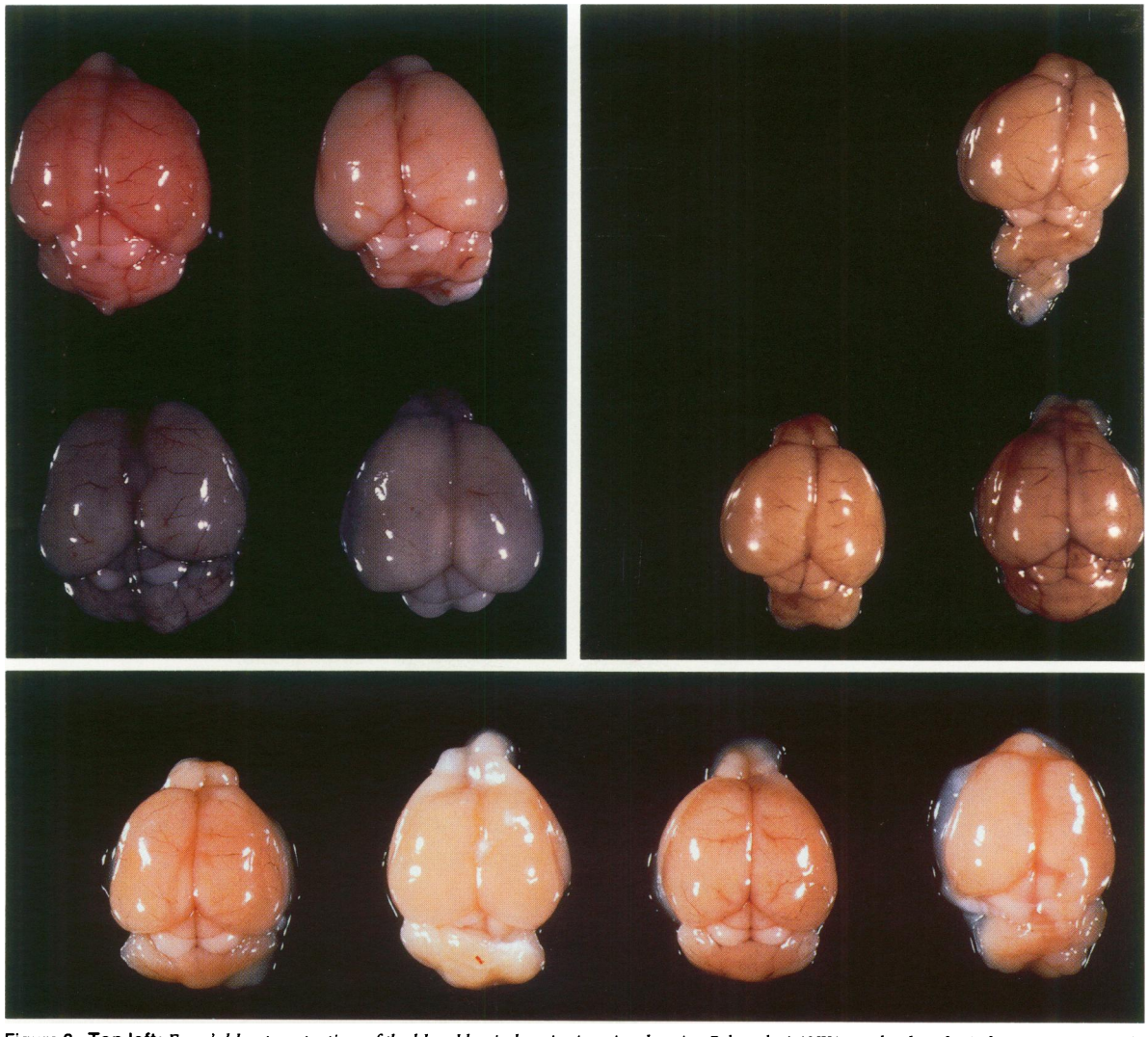

Figure 3. Top left: *Evan's blue penetration of the blood-brain barrier in mice showing* P. berghei *ANKA cerebral malaria but not in controls.* **Top right:** *Absence of Evan's blue penetration of the blood-brain barrier in terminal* P. vinckei-*infected mice.* **Bottom:** *Absence of Evan's blue penetration of the blood-brain barrier in* P. vinckei-*infected mice (35–40% parasitemia) given 5 μg r hu TNF/day for 2 days (top) and in saline injected controls (bottom).* **Next page:** *Absence of Evan's blue penetration of the blood-brain barrier in* P. berghei *ANKA-infected mice (3–4% parasitemia) given 3.75 (top) or 1.88 (middle) μg r mu TNF or saline (bottom) IV on 2 consecutive days.*

accompanied by neurologic symptoms or hemorrhage. The histologic microphotographs selected are representative of repeated experiments using both r mu and r hu TNF.

Integrity of Blood–Brain Barrier

We first confirmed an earlier study[18] that demonstrated movement of Evan's blue, which is excluded from normal murine brains, across the blood–brain barrier in mice showing signs of *P. berghei* ANKA cerebral malaria (Figure 3, top left). In contrast, mice severely ill from *P. vinckei,* which have higher circulating levels of TNF,[17] do not, by this criterion, lose integrity of their blood–brain barriers (Figure 3, top right).

To further test the possibility that high circulating levels of TNF would allow passage of Evan's blue across the blood–brain barrier, we injected 5 μg r hu TNF, or 2.25 μg r mu TNF, intravenously on 2 consecutive days into mice carrying 35% to 40% parasitemia of *P. vinckei* on the day of the first injection. These are the highest doses of these two cytokines such mice could withstand, yet 5 hours after the second injection the blood–brain barrier was, as judged by Evan's blue exclusion, still intact (Figure 3, bottom). In further experiments, mice carrying low (3% to 4%) parasitemias of *P. berghei* ANKA did not lose the capacity to exclude Evan's blue after 1.88 or 3.75 μg r mu TNF had been administered intravenously on 2 con-

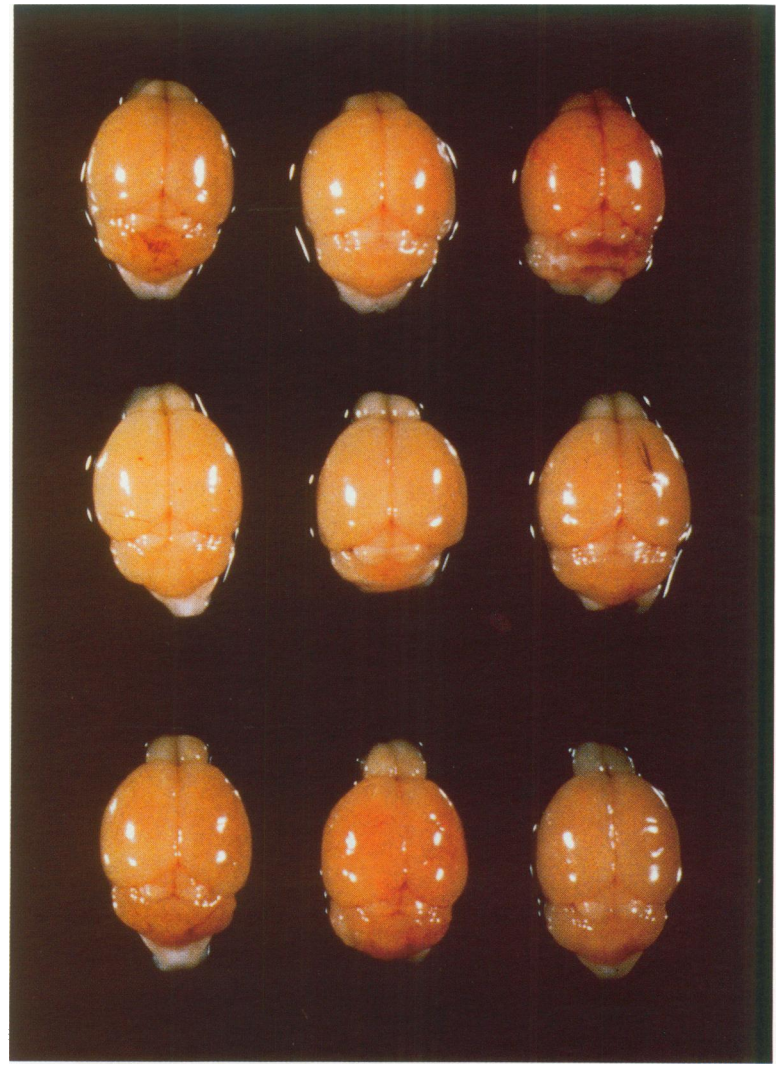

Figure 3. *(Continued).*

secutive days (Figure 3, this page). By this stage of the infection, as evidenced by controls, these mice were within 1 to 2 days of losing integrity of the blood–brain barrier from the effects of the *P. berghei* ANKA infection alone.

Liver Histology

Midzonal liver necrosis is routinely present in mice severely ill from *P. vinckei,*[19] and can be induced early in the infection by either LPS[19] or r hu TNF.[3] As with hypoglycemia, midzonal necrosis is absent in mice exhibiting *P. berghei* ANKA cerebral malaria[1] (Figure 4a). In the present experiments, midzonal necrosis was evident in all parasitized mice given r hu or r mu TNF, provided that, as reported earlier,[19] at least 8 hours had elapsed from the first injection of TNF to the time the organ was fixed. It was most severe in *P. berghei* ANKA and *P. vinckei*–infected mice given daily intravenous injections of 2.5 μg r mu TNF (Figures 4b and 4c), yet was absent in saline-injected *P. berghei* ANKA controls when they developed cerebral symptoms 1 to 2 days later (not shown). The histologic microphotographs are again representative of repeated experiments using both r mu and r hu TNF.

Blood Glucose and Plasma TNF Levels

In keeping with our earlier experience,[3] mice infected with *P. vinckei* routinely developed severe hypoglycemia as the infection reached its final stages (data not shown). Terminally ill *P. berghei* ANKA-infected mice were, however, not hypoglycemic, but had normal blood glucose levels (11.72 ± 4.17 mmol/l, n = 24). In contrast, all of the parasitized mice given either r mu or r hu TNF parenterally became hypoglycemic, the degree depending on

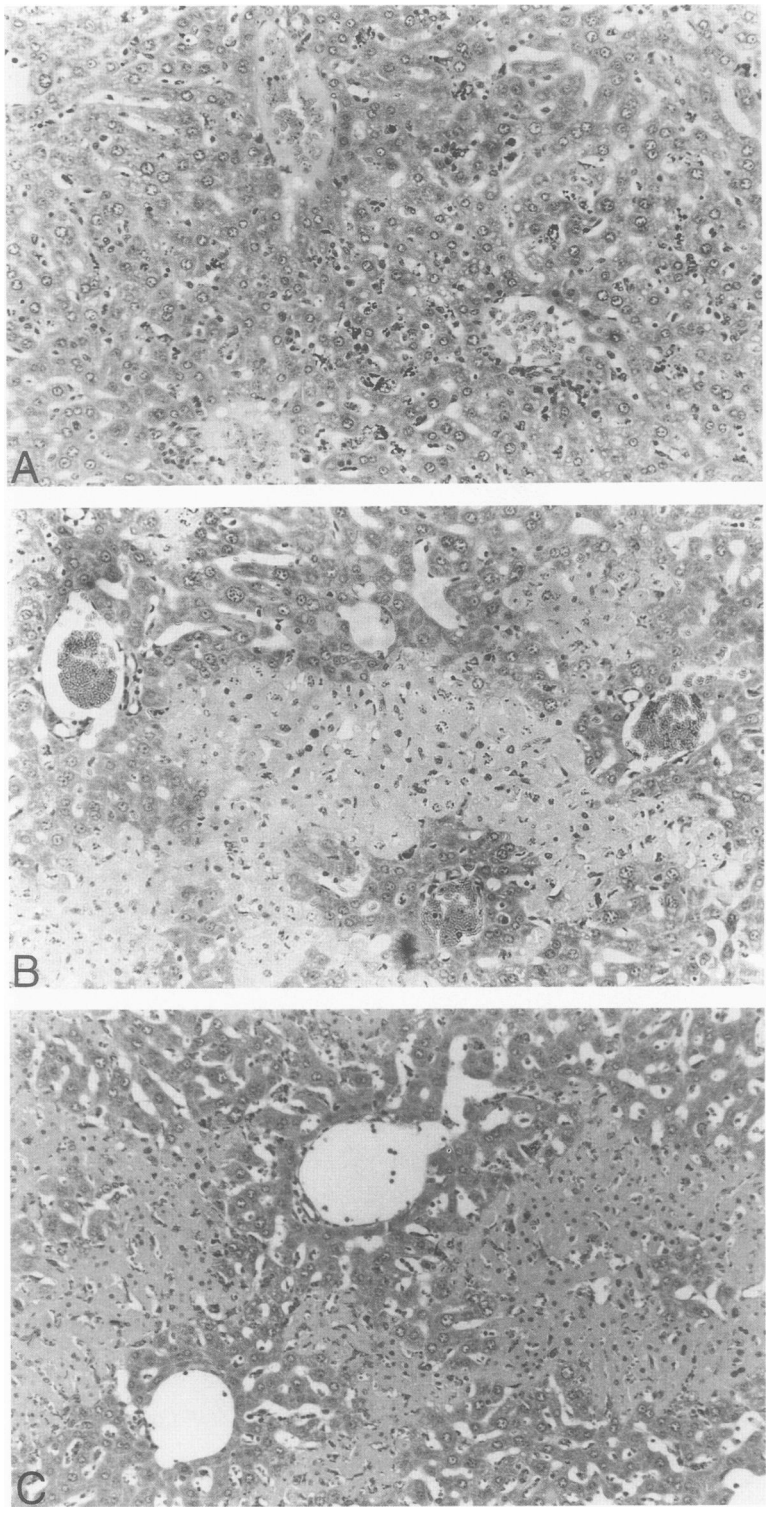

Figure 4. A: *Liver section from a mouse showing* P. berghei *ANKA cerebral malaria. Midzonal necrosis absent, (H&E, original magnification ×100).* **B:** *Liver section from a* P. berghei *ANKA-infected mouse (3–4% parasitemia) given 2.5 μg r mu TNF IV on 2 consecutive days. Midzonal necrosis present, (H&E, original magnification ×100).* **C:** *Liver section from a* P. vinckei*-infected mouse (20% parasitemia) given 2.5 μg r mu TNF IV on 2 consecutive days. Midzonal necrosis present, (H&E, original magnification ×100).*

the dose of TNF administered and the general level of illness that was induced (Figure 5). This reflects our earlier more limited experience.[3] In neither case was murine cerebral malaria induced, as judged by clinical condition and by normal gross and histologic conditions of brains (not shown). Figure 6 shows that plasma TNF levels correlated with blood glucose level as undisturbed *P. vinckei* infections ran their course.

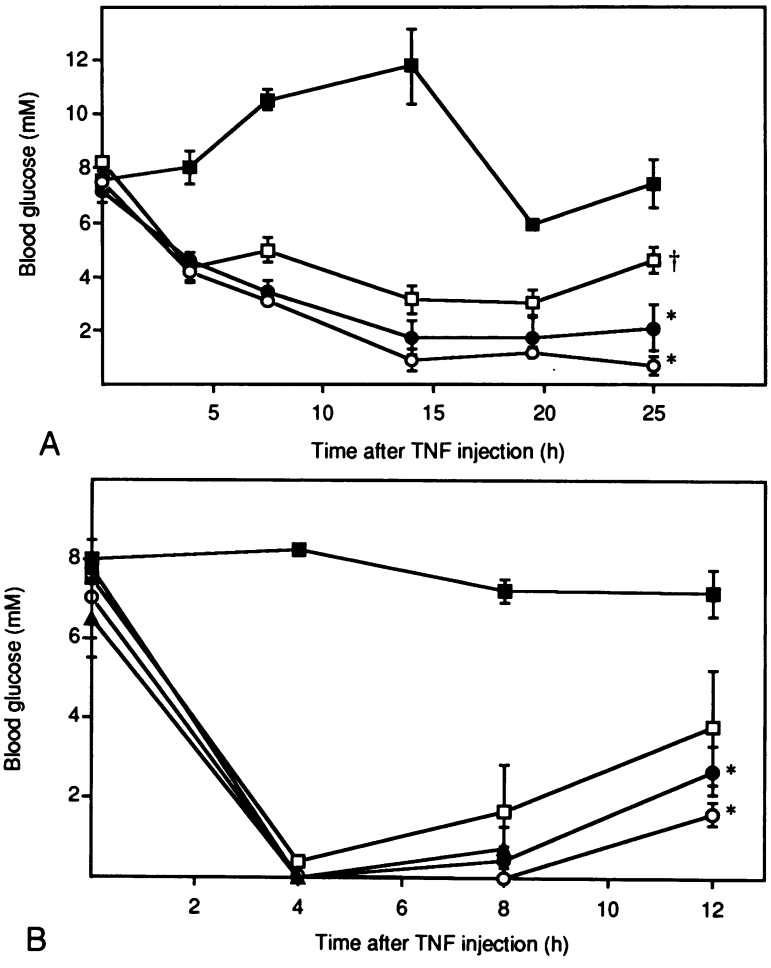

Figure 5. A: *Blood glucose levels in* P. berghei *ANKA-infected mice (4–8% parasitemia) given r mu TNF:* —■— *saline;* —□— *4.3 μg TNF;* —●— *8.5 μg TNF;* —○— *17.1 μg TNF. Symbols represent means ± SEM of four animals.* †*Significantly different from saline treated animals* $P < 0.05$; *$P < 0.01$. B: *Blood glucose levels in* P. vinckei-*infected mice (30–40% parasitemia) given r mu TNF:* —■— *saline;* —□— *0.4 μg TNF;* —●— *0.8 μg TNF;* —○— *1.6 μg TNF;* —▲— *3.2 μg TNF (N.B. all animals from this group were dead after 8 hours). Symbosl represent means ± SEM of four animals; similar results were obtained using r hu TNF (data not shown).* **Significantly different from saline treated animals,* $P < 0.01$.

Discussion

A polyclonal antibody raised against r mu TNF has been reported to prevent the cerebral lesions that *P. berghei* ANKA usually causes in CBA mice.[9] These authors made comparisons of serum TNF levels in these animals, with a number of other mouse malarias as controls (sampled at the time after infection at which cerebral malaria-susceptible mice first displayed neurologic signs) and concluded that the critical event determining the onset of

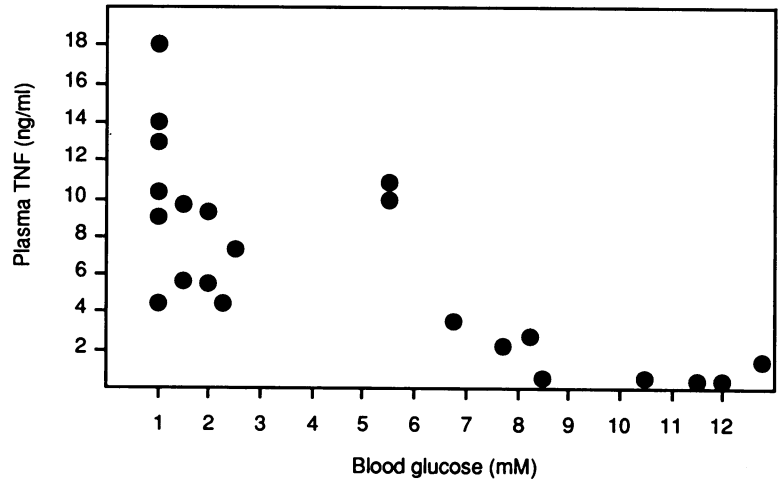

Figure 6. *Relationship between plasma TNF and blood glucose levels throughout undisturbed* P. vinckei *infections. Samples were taken throughout the full range of parasitemia values. Symbols represent individual mice.* $R = 0.75$; $P < 0.01$.

murine cerebral malaria was the amount of TNF released into the circulation. From common experience with these parasites, however, the parasite load in these control groups of mice would have been too low to cause illness at the time they were bled for serum.

We had earlier[3] been unable to reproduce mouse cerebral malaria by giving a single injection of r hu TNF to mice lightly infected with *P. vinckei*. Instead, we reproduced the systemic illness typical of *P. vinckei* and characterized by hypoglycemia and liver injury, neither of which occurs in *P. berghei* ANKA cerebral malaria. This led us to compare TNF levels in mice ill from *P. vinckei* and *P. berghei* ANKA infections: *P. vinckei*-infected mice proved to have consistently higher TNF levels at the time of onset of illness, even though they did not have cerebral symptoms.[17] Because TNF induces IL-6,[12] our present observation that serum IL-6 was considerably higher in mice ill from *P. vinckei* infection than in those exhibiting *P. berghei* ANKA cerebral malaria (Figure 1) is consistent with our earlier findings.[17]

If the concentration of TNF in serum controls the onset of murine cerebral malaria,[9] it should be possible to induce this condition by artificially raising serum levels with exogenous TNF. We have attempted this using both r hu and r mu TNF at a range of doses and timings, by intravenous and by controlled intraperitoneal release from osmotic pumps, and in mice primed with a range of malaria parasites. In no case were we successful: integrity of the blood–brain barrier was never breached (Figure 3c, d), and no brain hemorrhages or neurologic symptoms were observed (Figure 2b), even though we administered sufficient human or mouse TNF to cause midzonal liver necrosis (Figure 4b, c) and hypoglycemia (Figure 5). The larger doses of TNF required to cause hypoglycemia in *P. berghei* ANKA-infected mice (Figure 5a) do not reflect a difference between the effects of this parasite and of *P. vinckei* (Figure 5b), but in the parasitemia employed: sensitivity of the host to TNF increases markedly as parasitemia rises.[3] Endogenous TNF was undetectable in all infected mice in these experiments.

Midzonal liver necrosis and hypoglycemia are characteristic of both TNF toxicity and acute *P. vinckei* infection, and absent in *P. berghei* ANKA cerebral malaria. For these reasons, summarized in Table 1,[6,20,21] we propose that the onset of murine cerebral malaria is not linked to high serum TNF levels.

In addition, were the onset of murine cerebral malaria a part of systemic TNF toxicity,[9] one would expect, in the light of a recent report by Starnes et al,[22] that it could be prevented by administering a neutralizing antibody directed against IL-6. This is at variance with recent experience, in which such passive immunization, potent enough to reduce immunoglobulin levels, failed to influence the onset or progression of P. berghei ANKA cerebral malaria.[10] It seems more likely that TNF will prove to have an essential role in the local cellular events within the cerebral vessels, a possibility we are at present examining through *in situ* hybridization to identify cytokine mRNA in these lesions. If the production of TNF proves to be limited to these locally adhering monocytes, the arguments of TNF-based similarities between murine and human cerebral malaria[11,23] are considerably weakened, because monocytes rarely adhere to cerebral vessels in the human disease.

Several groups have now published correlations between serum TNF levels and disease severity in falciparum malaria,[7,24–26] but only the most recent of these[26] has provided the information that coma status, excluding hypoglycemia, correlates with TNF. Neurologic changes during falciparum malaria do not occur in isolation, as in murine cerebral malaria, but rather as part of a generalized illness, often involving hypoglycemia in African children[24,27,28] and a multiplicity of other systemic dysfunctions in adults.[7] The outstanding correlate with TNF to emerge from the human data is hypoglycemia,[24,26] a condition absent in P. berghei ANKA cerebral malaria and present, as well as correlating with TNF levels, in P. vinckei infections (Figure 6). Likewise levels of TNF[6] and IL-6 (Figure 1) both correlate with disease severity in *P. vinckei,* as they do in falciparum malaria.[7]

Although a form of cerebral malaria is present in *P. berghei* ANKA, it is associated with a local and unique lesion, with cellular components different from those present in the human disease, and without the systemic

Table 1. *Comparison of Characteristic Parameters of TNF Toxicity, Terminal* P. berghei *ANKA and* P. vinckei *Infections, and Human Falciparum Malaria*

	Serum TNF	Plasma IL-6	Evan's Blue permeability to brain	Adherent monocytes in brain vessels	Brain hemorrhage	Hypoglycemia	Hepatic damage
TNF toxicity	NA	+++[20]	−*	±*	−*	+*	+[3]
P. vinckei	+++[6]	+++*	−*	−*	−*	+*	+[3]
P. falciparum	+++[7,24,26]	+++[7,†]	NA	−	±‡	+[24,26]	+[21]
P. berghei ANKA	+[17]	+*	+[18]	+*	+[1]	−[17]	−[1]

* See Results.
† Clark et al. submitted manuscript.
‡ Certain fatal cases only.

changes that precede and accompany cerebral malaria in man.[2] Additionally the blood–brain barrier remains largely intact in severe *P. vinckei* mouse malaria (Figure 3b), in human cerebral malaria,[29] and also in systemic TNF toxicity (Figure 3c, d), at least in the mouse. This is in contrast to *P. berghei* ANKA cerebral malaria, where, in the absence of the systemic changes that characterize *P. vinckei* and *P. falciparum* infections (Table 1), the blood–brain barrier is routinely breached[18] (Figure 3a).

Taken together, these results are not consistent with earlier reports linking murine cerebral malaria with circulating levels of TNF,[10] although they are compatible with such an association in human cerebral malaria.[26] This condition is associated with systemic illness, and the blood–brain barrier is (in contrast to the changes seen in *P. berghei* ANKA infections) generally not breached.[29] We also conclude that the higher levels of circulating cytokines, and associated histologic and biochemical changes seen in *P. vinckei* malaria but not in *P. berghei* ANKA infections, make the former closer in concept to falciparum malaria and thus the more useful model through which to understand the human disease.

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