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Cytokine Profile Suggesting that Murine Cerebral Malaria Is an Encephalitis

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Cerebral malaria (CM) remains a poorly understood and life-threatening complication of malaria caused by the parasite *Plasmodium falciparum*. The discovery that murine CM caused by *Plasmodium berghei* ANKA and human CM are both characterized by production of inflammatory cytokines, especially tumor necrosis factor alpha (TNF-α), led to a revival of the suggestion that *P. berghei* CM may have value as a model of the human disease. In this study, quantitative reverse transcription-PCR was used to measure levels of message for 18S rRNA of *P. berghei* and 10 cytokines in the brains, livers, and spleens of mice during the induction and course of CM. A coordinated increase in RNA of parasite and proinflammatory cytokines was observed in the brains of mice in parallel with onset of CM. Levels of message for parasite, TNF-α, and gamma interferon increased in the brains of mice from day 5 to death on day 7. These changes were observed only in the brain, and message for other cytokines remained near baseline levels. This demonstrated that parasite sequestration does take place in the brains of mice with CM. Histologically, CM was characterized by widespread damage to the microvasculature in the brain with focal infiltration of inflammatory cells. The pattern of cytokine production in the brain is characteristic of other murine encephalitides.

The pathogenesis of cerebral malaria (CM) remains controversial despite nearly 100 years of research. According to the World Health Organization, the incidence of the disease is rising, and it currently causes 3,000 to 5,000 deaths/day, primarily in children. The spread of the resistance of parasites to antimicrobials and the resistance of malaria-carrying mosquitoes to insecticides is confounding efforts to control the disease, adding urgency to the need for new therapeutic approaches.

All symptoms of malaria are caused by parasites in erythrocytes (28). In general, children with CM have high levels of parasitemia. However, some tolerate this parasitemia with only mild symptoms, while others succumb to low-level parasitemia. This suggested that CM may be an immunopathology resulting from the host response to infection (3, 24). Early investigators described two characteristic pathologic changes in CM: sequestration of parasitized erythrocytes in small vessels of the brain and endothelial-cell damage ranging from swelling to microvascular necrosis (43). Hemorrhage, thrombosis, and mononuclear-cell inflammation were also reported (43). Sequestration of erythrocytes has consistently been emphasized in the human disease. It has been reported that in the mouse model, monocytes rather than parasitized erythrocytes are the predominant cells sequestered in the brain. This has led to frequent statements that *Plasmodium berghei* ANKA CM is not a good model of the human disease (25, 28). However, the histopathologic changes of both human and murine CM vary among individuals, and sequestration in both diseases has been attributed to expression of receptors on endothelial cells. Furthermore, some investigators report that the murine model can duplicate the pathologic changes of the human disease rather well (7, 30–33). Many uncertainties still remain.

Recent demonstrations that tumor necrosis factor alpha (TNF-α) is involved in the pathogenesis of both human and murine CM has rekindled interest in murine models (3, 14, 18, 20, 33). Multiple investigators have found that patients with CM are more likely than those with uncomplicated malaria to have elevated levels of TNF and TNF receptors in their circulation and that the levels of TNF predict outcome (17, 22, 27). Also, phospholipids of *Plasmodium falciparum* induce production of TNF by cells in culture nearly as effectively as lipopolysaccharide (21). Parasites isolated from children with CM were reported to be more likely to induce high TNF levels from cultured cells in vitro than isolates from children with uncomplicated malaria fever (3). Finally, children with a high level of promoter for TNF were reported to be more likely to develop CM than others (26). The data relating murine CM to TNF is even stronger. TNF production in brain tissue has been demonstrated by immunohistochemistry and reverse transcription-PCR (RT-PCR) (14, 15). Injection of TNF into genetically resistant infected mice has been reported to trigger development of CM, while injection of anti-TNF antibodies prevents CM (15).

TNF does not act alone in the induction of CM. There is increasing evidence that it acts in concert with gamma interferon (IFN-γ) and other cytokines (14, 36). While these investigations have established that TNF and other cytokines contribute to the pathogenesis of CM, they have not determined why the brain is particularly susceptible. The development of quantitative RT-PCR which uses a highly sensitive bioluminescent tag makes it possible to measure parasites and markers of inflammation in tissue with a precision not previously possible (1, 37). We used this method with histopathologic examination to investigate cytokines in the brains, spleens, and livers of mice developing CM. The results demonstrate coordinated production of message for proinflammatory cytokines and se-

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questration of parasite RNA in the brains of mice with focal infiltration of inflammatory cells in a pattern characteristic of encephalitis.

Animals, parasites, and infection. Twelve-week-old pathogen-free female C57BL/6J and A/J mice (Jackson Laboratory, Bar Harbor, Maine) were infected intravenously with 10^4 *P. berghei* ANKA-parasitized erythrocytes from homologous donors that had been infected with frozen stock maintained in Glycerolyte 57 solution (Baxter Health Corp., Deerfield Ill.) at −70°C in 200-μl aliquots (5). Age- and strain-matched naive mice were used as controls. Parasitemia was assessed from Giemsa-stained thin smears of tail blood prepared at least every other day postinfection, and the smears were used to determine the percentage of parasitized erythrocytes. C57BL/6J mice developed paralysis, convulsions, and coma and died within 7 days of inoculation with parasitemias of less than 10%. A/J mice, in contrast, developed similar parasitemia for the first 7 days but survived several weeks longer without cerebral signs and died with anemia and overwhelming parasitemia of 60 to 80%.

Quantitative bioluminescent RT-PCR procedure. Blood (100-μl) or tissue (25-mg) samples from infected or control mice were collected, placed in RNAzol B (TelTest, Inc., Friendswood, Tex.), and frozen at −70°C. RNA was extracted, resuspended in diethylpyrocarbonate-treated water, quantitated spectrophotometrically, and stored at −70°C. RNA (1.0 μg) was reverse transcribed to cDNA in a 25-μl volume using reverse transcriptase (Life Technologies, Gaithersburg, Md.) (42). The resulting cDNA was diluted 1:8 prior to PCR amplification. Five microliters was further subjected to PCR.

Optimum cycles for PCR were determined empirically where detectable products were below saturating conditions during log-linear amplification. PCR denaturation, annealing, and elongation were carried out at 94, 54, and 72°C for 1, 1, and 2 min, respectively, with a PTC-100-96V thermal cycler (MJ Research, Watertown, Mass.). Primers (5′ to 3′) were as follows: for IFN-γ cDNA (399 bp), biotin-AAC-GCT-ACA-CAC-TGC-ATC-T (sense) and GAG-CTC-ATT-GAA-TGC-TTG-G(antisense); for TNF-α cDNA (254 bp), GAT-CTC-AAA-GAC-AAC-CAA-CTA-GTG (sense) and biotin-CTC-CAG-CTG-GAA-GAC-TCC-TCC-CAG (antisense); for interleukin-1β (IL-1β) cDNA (194 bp), GGA-ATG-ATG-ATG-ATA-ACC-TG (sense) and biotin-TTG-TCG-TTG-CTT-GGT-TCT-CCT (antisense); for *P. berghei* 18S rRNA (678 bp), ATC-AGC-TTT-TGA-TGT-TAG-GGT-ATT-G (sense) and GCG-GTA-ATT-CCA-GCT-CCA-A (antisense); and for β-actin cDNA (185 bp), TGT-TAC-CAA-CTG-GGA-CGA-CA (sense) and biotin-GGA-TGG-CTA-CGT-ACA-TGG-CT (antisense). The cycle number was 20 for β-actin and IL-1β and 25 for IFN-γ and TNF-α. The primers were synthesized at the microchemical facility at Emory University (Atlanta, Ga.) or Operon Technologies (Alameda, Calif.). cDNA samples were additionally subjected to 40 cycles of amplification and electrophoresis followed by ethidium bromide staining to confirm single-band products.

Quantitation of PCR product was accomplished by using the microplate bioluminescent assay previously described (1, 37, 38). Biotinylated PCR product (5 μl) was denatured for 5 min with 1.25 μl of denaturation buffer (1 M NaOH, 200 mM EDTA). Neutralization buffer (6.25 μl) (0.15 M Na_2HPO_4, pH 6.0) was added and allowed to react for 1 min. The sample was transferred to streptavidin-coated 96-well microplates (MicroCoat, Penzberg, Germany) containing 4 ng of digoxigenin (DIG)-labeled probe in hybridization buffer (62.5 mM Na_2HPO_4, 0.94 M NaCl, 94 mM citric acid, 10 mM $MgCl_2$, 0.125% Tween 20, 0.0625% bovine serum albumin, 15 mM NaN_3 [pH 6.5]). The probes were as follows: for IFN-γ cDNA, DIG-TCG-CCT-TGC-TGT-TGC-TGA; for IL-1β cDNA, CAG-CTG-CAC-TAC-AGG-CTC-CG-DIG; for TNF-α cDNA, CTC-TTC-AAG-GGA-CAA-GGC-TG-DIG; and for *P. berghei* 18S rRNA, TTG-GAG-CTG-GAA-TTA-CCG-C-DIG. Hybridization at 42°C for 1 h was followed by washing with 150 μl of SeaLite wash buffer (SeaLite Sciences, Inc., Norcross, Ga.). AquaLite-conjugated anti-DIG antibody (5 ng) (SeaLite Sciences, Inc.) was added for 30 min with agitation (180 rpm) at room temperature and washed. The bioluminescent reaction was initiated with AquaLite trigger solution (50 mM Tris, 10 mM calcium acetate, 15 mM NaN_3 [pH 7.5]) in an ML 3000 luminometer (Dynatech Laboratories, Inc., McLean, Va.), and light emission measured at 469 nm was integrated over 1.8 s to produce results in relative light units (RLU). Results on blood and tissue samples were normalized to β-actin (GAG-CAC-CCT-GTG-CTG-CTC-DIG), according to the method previously described for hypoxanthine phosphoribosyltransferase (39).

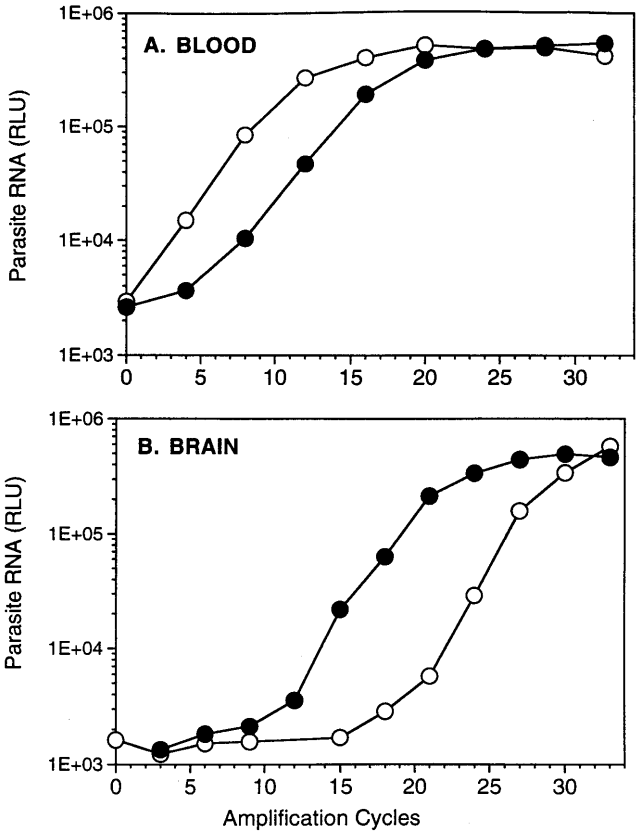


FIG. 1. *P. berghei* parasite message in blood and brain tissue of CM-susceptible and CM-resistant mice. Total RNA was isolated from blood or brain tissue of CM-susceptible (C57BL/6J) mice (solid circles) at 7 days or CM-resistant (A/J) mice (open circles) at 20 days. Message for 18S rRNA *P. berghei* was quantitated by RT-PCR followed by ultrasensitive bioluminescent detection of product. The results of increasing PCR amplification from 0 to 33 cycles are shown to illustrate the performance of the assay during the log-linear phase of PCR amplification. This experiment was repeated four times with equivalent results.

Quantitation of *P. berghei* message in blood, brain, spleen, and liver. Parasite-specific RNA was measured in blood and brains of *P. berghei*-infected CM-susceptible (C57BL/6J) mice on day 7 and CM-resistant (A/J) mice on day 20 when they became moribund (Fig. 1A). At the present stage of development, the method is not sufficiently reproducible to provide

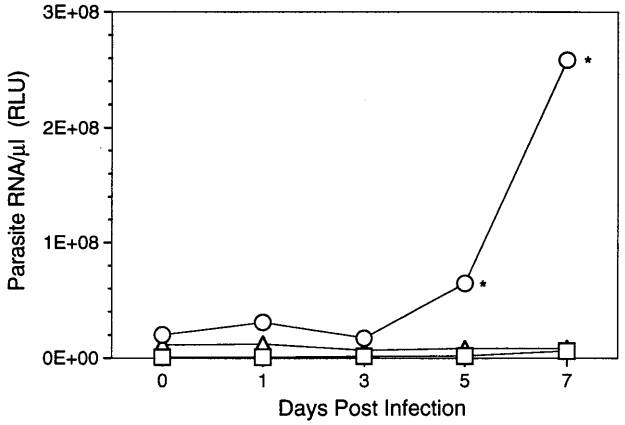


FIG. 2. *P. berghei* parasite message in brain, spleen, and liver during induction of CM. *P. berghei* message was measured in the brains (circles), spleens (squares), and livers (triangles) during the course of CM in C57BL/6J mice. Measurements were made by using 20 PCR cycles as optimized in preliminary studies. The results are the mean (n = 3, standard deviation, <10%) RLU per microliter of blood volume in each tissue (40). Day 0 levels represent a background of <1,200 RLU. The increase in parasite RNA in the brain compared to the level in the liver or spleen on days 5 and 7 was statistically significant at a P value of <0.01 (*) by the nonparametric Mann-Whitney test.

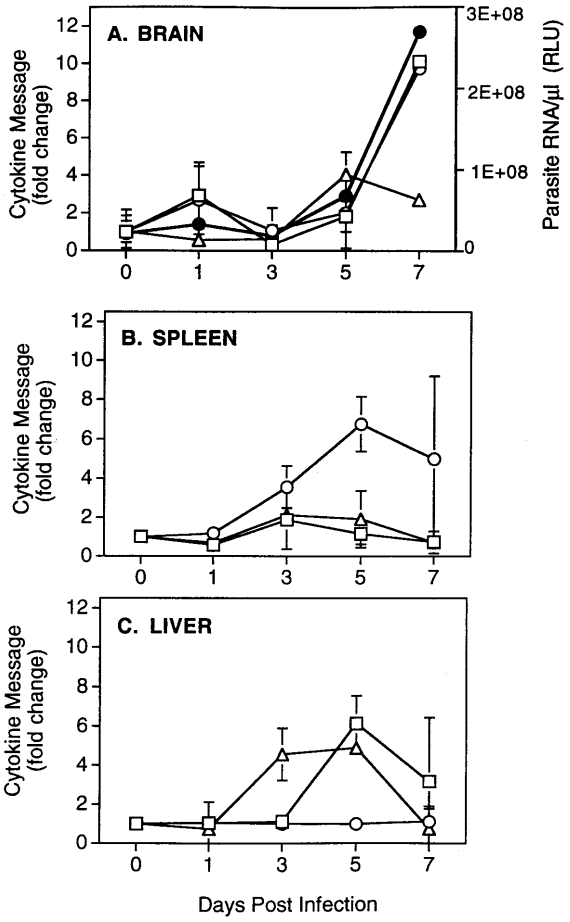


FIG. 3. Cytokine message in brain, spleen, and liver during induction of CM. Message for IFN-γ (open circles), IL-1β (triangles), TNF-α (squares), and *P. berghei* parasites (solid circles) from tissues of infected mice was measured with bioluminescent RT-PCR. The results are presented as mean fold change compared to noninfected control mice for brain (A), spleen (B), and liver (C). (n = 3 or 4; data are means ± standard deviations).

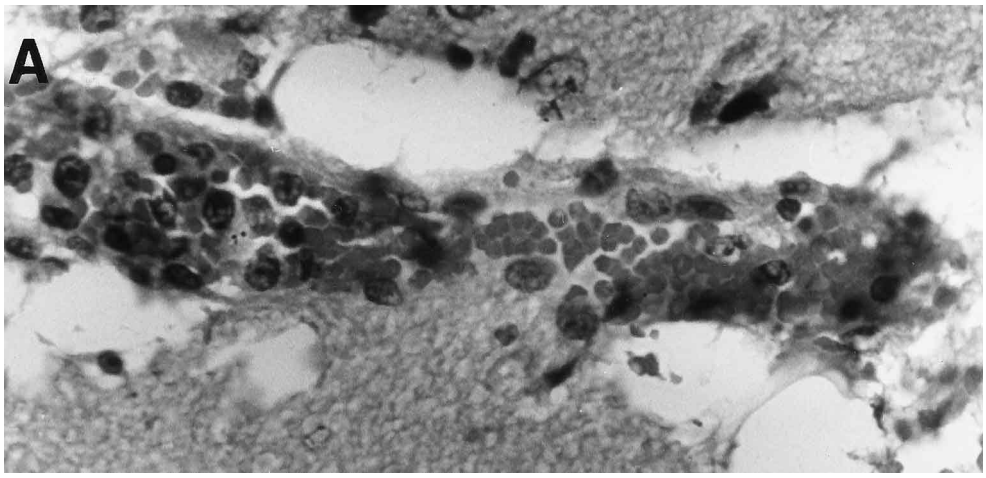
confidence in reporting results of RT-PCR analysis as absolute counts of parasites or parasitized erythrocytes. Nevertheless, in several experiments we calculated that between 4 and 7 RLU were detected for each parasitized erythrocyte. The most accurate quantitation of RNA was the ordinal difference between the linear portions of the curves (the difference between their x intercepts) derived from linear regression. By this analysis, the parasite RNA level was 31-fold higher in the blood of A/J than C57BL/6J mice. This was consistent with parasite counts on Giemsa-stained smears. However, the pattern was reversed in the brains of the same animals: the parasite RNA level was 202-fold higher in C57BL/6J mice than in A/J mice (Fig. 1B). Consequently, ~6,000-fold (31 × 202) more parasites became sequestered in the brains of C57BL/6J mice in parallel with the onset of CM than could be accounted for by parasitemia. In longitudinal studies of susceptible mice, parasite RNA increased rapidly in brain tissue from 5 days after infection until death (Fig. 2). The levels of parasite RNA in livers and spleens of these animals did not show a similar rapid increase during this time. It is important to point out that the demonstration of parasite message in the brains of mice does not imply that the mechanisms of sequestration are similar to those in human CM.

Quantitation of cytokine message in brain, spleen, and liver. CM in mice has been associated with elevated levels of TNF (9, 19). However, injections of TNF fail to induce cerebral signs, suggesting that TNF is produced locally in the brains of mice with CM (9, 14). In order to determine if this is a manifestation of localized encephalitis or systemic disease, we measured message for proinflammatory cytokines in the brains, livers, and spleens of mice during the course of disease. In the brain, levels of RNA for TNF-α and IFN-γ rose in parallel with parasite RNA levels (Fig. 3A). This was associated with an earlier (day 5) and smaller increase in levels of RNA for IL-1β. These three proinflammatory cytokines are known to act synergistically (9, 14). No significant induction of message for IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, or granulocyte-macrophage colony-stimulating factor was detected in the brains of these mice during the course of disease. This data is consistent with earlier reports that message for TNF, IFN, and IL-1a is upregulated in the brains of mice with CM (13).

The cytokine profile in other organs was different and did not correlate with the development of CM. In the spleen, IFN-γ message increased and persisted throughout the course of infection, but that for TNF-α and IL-1β remained near baseline (Fig. 3B). In the liver, IL-1β and TNF-α expression increased, while that of IFN-γ remained near baseline (Fig. 3C). No significant induction of message for IL-2, IL-4, or IL-12 was detected in the livers or spleens of these animals.

Since encephalitis is defined as "inflammation of the brain" (4), these results demonstrate that murine CM is an encephalitis characterized by sequestration of parasites and production of message for proinflammatory cytokines in the brain. Mice die of the encephalitis while parasitemia is at levels that produce no detectable symptoms in CM-resistant mice. The pattern of cytokine production in these mice is similar to that reported to occur in experimental allergic encephalomyelitis and in the encephalitis produced by lymphocytic choriomeningitis virus (8, 29, 34). In both of these diseases, the expression of RNA message in brain tissue for IFN-γ, IL-1β, and TNF-α correlates with the progression and severity of disease.

Gross and histologic examination of brains of mice with CM



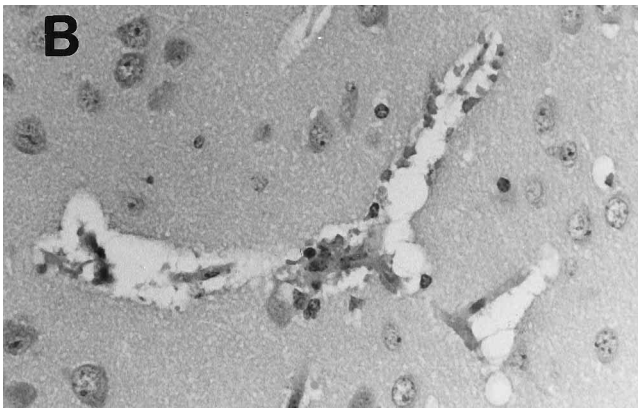


FIG. 4. Histopathology of the brains of mice with CM. (A) Cerebral blood vessels were packed with mononuclear cells and parasitized erythrocytes. (B) Cerebral vessel showing fragmentation and death of endothelial cells without infiltration of mononuclear or other inflammatory cells (hematoxylin and eosin stain). Magnifications, ×400 (A) and ×190 (B).

further defined the disease. Cross sections of the brains of mice with CM revealed petechial hemorrhages in the deep white matter of the cerebral and cerebellar cortexes. No gross abnormalities were visible in the gray matter of the outer cortex. Sections of brain were fixed in 10% neutral buffered formalin and processed for microscopic analyses. The typical inflammatory lesions of murine CM, consisting of accumulation of mononuclear cells with occasional parasitized erythrocytes in cerebral blood vessels, were observed (Fig. 4A). However, these changes were restricted to focal areas of the cortex. A more widespread change was swelling and degeneration of endothelial cells with surrounding edema. These changes began by day 3 and progressed by day 7 to the point of widespread death and fragmentation of the cerebral microvasculature (Fig. 4B). The separation of areas of inflammation from those of microvascular destruction suggests that the proinflammatory cytokine message detected in the brains of these mice might be produced by brain cells (microglial cells or astrocytes) instead of or in addition to monocytes (11, 23). Nevertheless, coordinated synthesis of three proinflammatory cytokines (TNF, IFN, and IL-1) is probably important since they have been reported to act synergistically to produce deleterious effects, including killing of endothelial cells.

The possible similarities of murine CM and human CM require comment. Virtually all investigators of the human disease have reported endothelial-cell damage in addition to obstruction of the microcirculation by parasitized erythrocytes (2, 33, 41). Some investigators report that there is little inflammatory response (28). Others report that monocytes can be more prevalent in blood vessels than parasitized erythrocytes and that obstruction of cerebral vessels with parasitized erythrocytes is not required for the development of brain lesions in humans (7). Toro and Roman reported that CM has histologic features reminiscent of postvaccinal or parainfectious encephalomyelitis (40). Swelling, degeneration, and death of microvascular endothelial cells with edema, hemorrhage, and thrombosis have also been reported by many investigators of human CM.

The hypothesis that CM is an encephalitis characterized by local production of proinflammatory cytokines in the brain could explain many of its manifestations. TNF can induce rapid onset and rapidly reversible increases in permeability of endothelial cells associated with edema of the brain (10). This might explain the rapid onset of CM and total recovery typical of survivors (12, 28). TNF also induces generation of monocyte chemotactic factors by endothelial cells and activation of the adhesion molecules ICAM-1 and E selectin (6, 16). Sequestration of *P. falciparum*-parasitized erythrocytes is thought to involve binding to these receptor molecules via surface knobs (2). This binding occurs in many organs but is greatly increased in the brain in CM, suggesting local activation of receptors (35). The mechanism of binding of *P. berghei* erythrocytes is unknown, although, like *P. falciparum*, it preferentially involves mature parasite forms (43). Finally, TNF can produce death of endothelial cells, focal vascular necrosis, hemorrhage, and thrombosis (41). Among individuals, as among strains of mice, the relative preponderance of manifestations varies. However, the common pathway may well be widespread activation and/or damage of endothelial cells by locally produced proinflammatory cytokines, specifically TNF and IFN acting in concert.

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REFERENCES

1. **Actor, J. K., M. Olsen, L. A. Boven, N. Werner, N. L. Stults, R. L. Hunter, and D. H. Smith.** 1996. A bioluminescent assay using AquaLite for RT-PCR amplified RNA from mouse lung. J. NIH Res. **8:**62.
2. **Aikawa, M.** 1988. Human cerebral malaria. Am. J. Trop. Med. Hyg. **39:**3–10.
3. **Allan, R. J., P. Beattie, C. Bate, M. B. Van Hensbroek, S. Morris-Jones, B. M. Greenwood, and D. Kwiatkowski.** 1995. Strain variation in tumor necrosis factor induction by parasites from children with acute falciparum

malaria. Infect. Immun. **63:**1173–1175.
4. **Anonymous.** 1992. American heritage electronic dictionary, 3rd ed. Houghton Mifflin Company, Boston, Mass.
5. **Barnwell, J. W., R. J. Howard, H. G. Coon, and L. H. Miller.** 1983. Splenic requirement for antigenic variation and expression of the variant antigen on the erythrocyte membrane in cloned *Plasmodium knowlesi* malaria. Infect. Immun. **40:**985–994.
6. **Berman, J. W., M. P. Guida, J. Warren, J. Amat, and C. F. Brosnan.** 1996. Localization of monocyte chemoattractant peptide-1 expression in the central nervous system in experimental autoimmune encephalomyelitis and trauma in the rat. J. Immunol. **156:**3017–3023.
7. **Boonpucknavig, V., S. Boonpucknavig, R. Udomsangpetch, and P. Nitiyanant.** 1990. An immunofluorescence study of cerebral malaria. Arch. Pathol. Lab. Med. **114:**1028–1034.
8. **Campbell, I. L., M. V. Hobbs, P. Kemper, and M. B. Oldstone.** 1994. Cerebral expression of multiple cytokine genes in mice with lymphocytic choriomeningitis. J. Immunol. **152:**716–723.
9. **Clark, I. A., S. Ilschner, J. D. MacMicking, and W. B. Cowden.** 1990. TNF and *Plasmodium berghei* ANKA-induced cerebral malaria. Immunol. Lett. **25:**195–198.
10. **Claudio, L., J. A. Martiney, and C. F. Brosnan.** 1994. Ultrastructural studies of the blood-retina barrier after exposure to interleukin-1 beta or tumor necrosis factor-alpha. Lab. Invest. **70:**850–861.
11. **Corsini, E., A. Dufour, E. Ciusani, M. Gelati, S. Frigerio, A. Gritti, L. Cajola, G. L. Mancardi, G. Massa, and A. Salmaggi.** 1996. Human brain endothelial cells and astrocytes produce IL-1 beta but not IL-10. Scand. J. Immunol. **44:**506–511.
12. **Daroff, R., J. Deller, A. Kastl, and W. Blocker.** 1967. Cerebral malaria. JAMA **202:**119–125.
13. **de Kossodo, S., and G. E. Grau.** 1993. Profiles of cytokine production in relation with susceptibility to cerebral malaria. J. Immunol. **151:**4811–4820.
14. **de Kossodo, S., and G. E. Grau.** 1993. Role of cytokines and adhesion molecules in malaria immunopathology. Stem Cells **11:**41–48.
15. **de Kossodo, S., and G. E. Grau.** 1997. Cytokines and malaria: for better or for worse, p. 459–476. *In* D. G. Friedland and J. S. Remick (ed.), Cytokines in health and disease, 2nd ed. Marcel Dekker, Inc., New York, N.Y.
16. **Elfont, R. M., D. E. Griffin, and G. W. Goldstein.** 1995. Enhanced endothelial cell adhesion of human cerebrospinal fluid lymphocytes. Ann. Neurol. **38:**405–413.
17. **Grau, G. E.** 1992. Essential role of tumor necrosis factor and other cytokines in the pathogenesis of cerebral malaria: experimental and clinical studies. Verh. K. Acad. Geneeskd. Belg. **54:**155–175.
18. **Grau, G. E., L. F. Fajardo, P. Piguet, B. Allet, P. Lambert, and P. Vassalli.** 1987. Tumor necrosis factor (cachectin) as an essential mediator in murine cerebral malaria. Science **237:**1210–1212.
19. **Grau, G. E., and J. N. Lou.** 1995. Experimental cerebral malaria: possible new mechanisms in the TNF-induced microvascular pathology. Soz- Praeventivmed. **40:**50–57.
20. **Grau, G. E., T. E. Taylor, M. E. Molyneux, J. J. Wirima, P. Vassalli, M. Hommel, and L. P. Lambert.** 1989. Tumor necrosis factor and disease severity in children with falciparum malaria. N. Engl. J. Med. **320:**1586–1591.
21. **Jakobsen, P. H., C. A. Bate, J. Taverne, and J. H. Playfair.** 1995. Malaria: toxins, cytokines and disease. Parasite Immunol. **17:**223–231.
22. **Kern, P., C. J. Hemmer, H. Gallati, S. Neifer, P. Kremsner, H. Dietrich, and F. Porzsolt.** 1992. Soluble tumor necrosis factor correlate with parasitemia and disease severity in human malaria. J. Infect. Dis. **166:**930–934.
23. **Lafortune, L., J. Nalbantoglu, and J. P. Antel.** 1996. Expression of tumor necrosis factor alpha (TNF alpha) and interleukin 6 (IL-6) mRNA in adult human astrocytes: comparison with adult microglia and fetal astrocytes. J. Neuropathol. Exp. Neurol. **55:**515–521.
24. **Mabeza, G. F., V. M. Moyo, P. E. Thuma, G. Biemba, D. Parry, H. Khumalo, P. Nyarugwe, S. Zulu, and V. R. Gordeuk.** 1995. Predictors of severity of illness on presentation in children with cerebral malaria. Ann. Trop. Med. Parasitol. **89:**221–228.
25. **MacPherson, G., M. Warrell, N. White, S. Looareesuwan, and D. Warrell.** 1985. Human cerebral malaria. Am. J. Pathol. **119:**385–401.
26. **McGuire, W., A. V. Hill, C. E. Allsopp, B. M. Greenwood, and D. Kwiatkowski.** 1994. Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malaria. Nature **371:**508–510.
27. **Molyneux, M. E., H. Engelmann, T. E. Taylor, J. J. Wirima, D. Aderka, D. Wallach, and G. E. Grau.** 1993. Circulating plasma receptors for tumour necrosis factor in Malawian children with severe falciparum malaria. Cytokine **5:**604–609.
28. **Oaks, S. C., V. S. Mitchell, G. W. Pearson, and C. C. Carpenter.** 1991. Malaria, obstacles and opportunities. National Academy Press, Washington, D.C.
29. **Okuda, Y., Y. Nakatsuji, H. Fujimura, H. Esumi, T. Ogura, T. Yanagihara, and S. Sakoda.** 1995. Expression of the inducible isoform of nitric oxide synthase in the central nervous system of mice correlates with the severity of actively induced experimental allergic encephalomyelitis. J. Neuroimmunol. **62:**103–112.
30. **Patnaik, J. K., B. S. Das, S. K. Mishra, S. Mohanty, S. K. Satpathy, and D. Mohanty.** 1994. Vascular clogging, mononuclear cell margination, and enhanced vascular permeability in the pathogenesis of human cerebral malaria. Am. J. Trop. Med. Hyg. **51:**642–647.
31. **Polder, T., C. Jerusalem, and W. Eling.** 1983. Topographical distribution of the cerebral lesions in mice infected with *Plasmodium berghei*. Tropenmed. Parasitol. **34:**235–243.
32. **Polder, T. W., C. R. Jerusalem, and W. M. Eling.** 1991. Morphological characteristics of intracerebral arterioles in clinical (*Plasmodium falciparum*) and experimental (*Plasmodium berghei*) cerebral malaria. J. Neurol. Sci. **101:** 35–46.
33. **Porta, J., A. Carota, G. P. Pizzolato, E. Wildi, M. C. Widmer, C. Margairaz, and G. E. Grau.** 1993. Immunopathological changes in human cerebral malaria. Clin. Neuropathol. **12:**142–146.
34. **Renno, T., J. Y. Lin, C. Piccirillo, J. Antel, and T. Owens.** 1994. Cytokine production by cells in cerebrospinal fluid during experimental allergic encephalomyelitis in SJL/J mice. J. Neuroimmunol. **49:**1–7.
35. **Riganti, M., E. Pongponratn, T. Tegoshi, S. Looareesuwan, B. Punpoowong, and M. Aikawa.** 1990. Human cerebral malaria in Thailand: a clinico-pathological correlation. Immunol. Lett. **25:**199–205.
36. **Rudin, W., N. Favre, G. Bordmann, and B. Ryffel.** 1997. Interferon-gamma is essential for the development of cerebral malaria. Eur. J. Immunol. **27:** 810–815.
37. **Siddiqi, A., V. M. Jennings, M. R. Kidd, J. K. Actor, and R. L. Hunter.** 1996. Evaluation of electrochemiluminescence and bioluminescence based assays for quantitating specific DNA. J. Clin. Lab. Anal. **10:**423–431.
38. **Stults, N. L., H. N. Rivera, R. T. Ball, and D. F. Smith.** 1995. Bioluminescent hybridization immunoassay for digoxigenin-labeled PCR products based on AquaLite, a calcium-activated photoprotein. J. NIH Res. **7:**74.
39. **Svetic, A., F. D. Finkelman, Y. C. Jian, C. W. Dieffenbach, D. E. Scott, K. F. McCarthy, A. D. Steinberg, and W. C. Gause.** 1991. Cytokine gene expression after in vivo primary immunization with goat antibody to mouse IgD antibody. J. Immunol. **147:**2391.
40. **Toro, G., and G. Roman.** 1978. Cerebral malaria: a disseminated vasculomyelinopathy. Arch. Neurol. **35:**271–275.
41. **Turner, G. D., H. Morrison, M. Jones, T. M. Davis, S. Looareesuwan, I. D. Buley, K. C. Gatter, C. I. Newbold, S. Pukritayakamee, and B. Nagachinta.** 1994. An immunohistochemical study of the pathology of fatal malaria. Evidence for widespread endothelial activation and a potential role for intercellular adhesion molecule-1 in cerebral sequestration. Am. J. Pathol. **145:**1057–1069.
42. **Wynn, T. A., I. Eltoum, A. W. Cheever, F. A. Lewis, W. C. Gause, and A. Sher.** 1993. Analysis of cytokine mRNA expression during primary granuloma formation induced by eggs of *Schistosoma mansoni*. J. Immunol. **151:**1430–1440.
43. **Yoeli, M.** 1976. Cerebral malaria—the quest for suitable experimental models in parasitic diseases of man. Trans. R. Soc. Trop. Med. Hyg. **70:**24–35.

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