Factors Contributing to the Development of Cerebral Malaria*

Cerebral malaria is one of the most serious manifestations of *Plasmodium falciparum* infection and causes a high mortality rate. Among American servicemen in Vietnam cerebral involvement occurred in cases of falciparum malaria in only 1.6 per cent;[1] but in Thailand,[2] seven per cent of all hospitalised cases of falciparum malaria show cerebral involvement. At present, the important factors contributing to the development of cerebral malaria are not precisely known. Several factors could be responsible: for the sake of convenience, these could be divided into two groups as follows:

1. Non-immunological factors.
   1.1 Plugging of cerebral blood vessels by parasitised cells.
   1.2 Endotoxin.
2. Immunological factors.
   2.1 Reduced humoral or cell-mediated immune responses.
   2.2 Immune complexes.
   2.3 Defects in non-specific immunity.

1. Non-immunological factors

1.1 Plugging of cerebral vessels by parasitised red blood cells

It has been commonly observed in autopsied cases that cerebral malaria patients had plugging of cerebral blood vessels by heavily parasitised red blood cells, vascular congestion, cerebral oedema, petechial haemorrhages and reactive gliosis giving rise to malarial (Durck) granuloma.[3-5] These autopsy findings may not represent entirely the actual pathological changes that would occur in living patients. Cerebral oedema, for example, was shown by Toro *et al*[5] to be present in 86 per cent of 19 autopsied cases, but in only two out of 10 patients studied by computerised tomography.[6] Plugging of cerebral blood vessels by parasitised cells has been reported invariably to be present in all of four cerebral malaria cases in the Gold Coast of Africa,[7] but in only four of 19 patients in Colombia.[5] Recent observation by D.A. Warrell, Faculty of Tropical Medicine, Bangkok, showed that among 45 fatal cases of cerebral malaria, all but one had infected erythrocytes in the cerebral vessels (personal communication). Studies in *Macaca mulatta* using *P. knowlesi* showed that obstruction of the cerebral blood flow occurred before the "plugging" of cerebral blood vessels,[8] which could be interpreted as suggesting that the plugging may not have been the primary cause of cerebral ischaemia. Nevertheless, plugging could contribute secondarily to greater cerebral damage. Vascular plugging by parasitised cells is most probably associated with some properties unique to parasitised erythrocytes thus enables them to bind to endothelial cells.[9] If plugging is an important factor contributing to the development of cerebral malaria, one should find differences among the isolates from patients with cerebral malaria (CM) and those with non-cerebral (NCM) malaria. Isolates from CM patients should bind more strongly to endothelial cells than those from NCM patients. Alternatively, NCM patients should have antibodies in their plasma to revert the binding of infected erythrocytes to endothelial cells; whereas CM patients do not have such antibodies.

1.2 Endotoxin

The possible role of endotoxin in the manifestation of severe malaria was suggested by Clark[10] who demonstrated that mice infected by *P. vinckei* and *P. berghei* were more susceptible to endotoxin, the lethal dose of which decreased several fold. Endotoxin has been demonstrated by Limulus amoebocyte lysate (LALT) in the plasma of mice infected with *P. berghei* and in the plasma of patients with falciparum malaria.[11] If endotoxin plays a role in the development of cerebral malaria, it should be possible to predict that significantly larger numbers of CM patients would be LALT-positive compared with NCM patients. In fact, this turns out to

*This investigation received financial support from the United Nations Development Programme/World Bank/World Health Organisation Special Programme for Research and Training in Tropical Diseases, and was done in cooperation with the Wellcome-Mahidol University-Oxford Tropical Medicine Research Programme.

be the case. In our recent study,[12] It was shown that on the day of admission, 67 per cent of 15 CM patients were LALT-positive, whereas only 21.4 per cent of NCM patients were positive. A follow-up study on cerebral malaria patients showed some variation in the LALT-positive rate from day to day with 86 per cent being positive on day 1 and 53.3 on day 3; all were negative on the day of discharge. Among the NCM cases, four out of eight patients with parasitaemia of over 90,000/mm^3 were LALT-positive whereas only two out of 20 patients with parasitaemia of less than 90,000/mm^3 were positive. All 30 controls were negative. It is not known from where the endotoxin in the plasma of the LALT-positive patients was derived. It could have come from the gut or from the parasites themselves, but not from the complicating Gram-negative bacteraemia. Our findings that endotoxaemia occurred in both CM and NCM cases with high parasitaemia and that endotoxaemia was not demonstrated in all CM cases suggest that endotoxin does not play a central role in the pathogenesis of cerebral malaria. The question is whether endotoxin has any auxiliary role in aggravating the symptoms in some CM cases, and if so, what is its mode of action? At present, the mode of action of endotoxin is not precisely known, but its effects are probably mediated by macrophages through the activation of their membrane receptors leading eventually to the release of prostaglandins.[13] Among the prostaglandins produced, only PGG_2 and not PGH_2 or PGE_2 can cause damage to the endothelial cells of cerebral blood vessels.[14] The fact that conversion of PGG_2 to PGH_2 causes the release of free oxygen radicals[15] and superoxide dismutase (SOD) reduces the number of PGG_2-mediated lesions[14] suggests that endothelial cell damage could be brought about by free oxygen radicals. It remains to be seen whether endothelial cell damage caused by free oxygen radicals has any role in the pathogenesis of cerebral malaria.

2. Immunological factors

2.1 Reduced humoral or cell-mediated immune responses.

2.1.1 Humoral immune responses.

2.1.1.1 Blood stages.

The observation that in Africa, cerebral malaria occurs most frequently in children of pre-school age, suggests that a lack of immunity favours its development.[16] In contrast with Africa, cerebral malaria occurs predominantly in adults in Chantaburi, eastern Thailand (D.A. Warrell, personal communication). The reason for this difference in the age groups affected is of interest and deserves further investigation. Our recent study[17] showed that CM patients had histories of fewer attacks of malaria in the previous five years than did NCM malaria patients. CM patients, on the whole, did not show defective humoral immune responses, since the initial seronegative rate and the mean initial indirect haemagglutinating (IHA) and immunofluorescent antibody (IFA) titres were not significantly different from those of NCM patients and the mean initial ELISA titre was even higher than that of the NCM cases. However, when CM patients were divided into two groups, i.e. complicated and uncomplicated cases of cerebral malaria, the complicated CM group showed evidence of having reduced humoral immune response, as the mean IHA titre was lower and the IHA seronegative rate was higher than those of the NCM patients and the uncomplicated CM group. Nevertheless, we do not considered that the reduced humoral immune response is the sole factor associated with the development of complicated malaria, as it was demonstrable only by IHA and not by the ELISA or IFA tests. It is also possible that the humoral immune response in CM patients may be directed against antigens which are irrelevant to protective immunity. To clarify this point, it would be desirable that tests allegedly associated with protective immunity be carried out. There are several candidates for such tests including Cohen's growth inhibition test[18] which acts by preventing re-invasion of infected cells by merozoites, radioimmunoprecipitation,[19] prevention of binding of infected red blood cells to endothelial cells[9] or melanoma cells[20] and clustering of extracellular merozoites.[21] Our growth inhibition test results showed that CM patients even had higher serum growth inhibitory activity than that of NCM patients.[17] This inhibitory activity was not mediated by IgG, and was associated with increased serum level of alpha-1 antichymotryspin.[22] Further investigation should be carried out comparing the inhibitory action of purified IgG from cerebral and non-cerebral malaria cases.

Radioimmunoprecipitation has been used to show that sera from people living in a highly endemic area of Africa exhibited more precipitating bands and/or more intense staining bands against the extract of metabolically labelled schizonts than did sera from patients experiencing their first malaria attack.[19] The molecular weights of the schizont-derived peptides which reacted with these sera were >200, 140, 105 and 82 kilodaltons.[19] In contrast, Brown *et al*[22] showed that IgG from immune Papua New Guinean sera with inhibitory activity against parasite growth reacted with the 96-kilodalton schizont antigen, whereas sera without growth inhibitory activity did not. In Thailand, it is not known which schizont antigenic peptides are more likely to be associated with protective immunity. Nevertheless, when this test was performed on 31 CM patients, 15 NCM patients and eight healthy controls, there were only minor differences in the number and intensity of the radioimmunoprecipitating bands with re-

gard to these two groups of patients, especially in the 135-, 103- and 71-kilodalton bands.[24] In general, our results appear to indicate that this test is inadequate for distinguishing between CM and NCM patients.

2.1.1.2 Sporozoites

Because of the non-cyclical nature of sporozoites, measurement of antibodies against them is considered to be a better indicator for the assessment of previous malaria exposure than is the measurement of antibodies against the blood stage. Cerebral malaria patients were shown to have mean anti-sporozoite antibody titre lower than that of NCM patients.[22] This finding indicates that CM patients had been exposed to malaria less frequently than NCM patients and thus should be less immune to malaria than NCM patients.

2.1.2 Cell-mediated immune response.

The role of cell-mediated immunity in CM and NCM patients has recently been reported.[26] It was shown that most CM patients have an impairment of the initial delayed cutaneous response to phytohaemagglutinin and soluble protein antigens, whereas in NCM cases this impairment was related directly to the degree of parasitaemia. On the other hand, lymphocyte proliferative responses to lectins in cerebral and non-cerebral cases were within the normal range. After successful anti-malaria therapy, rapid restoration of cell-mediated functions ensued. These results do not support any evidence in favour of a pre-existing cellular immune deficiency in relation to the development of cerebral malaria.[26]

2.2 Immune complexes.

There has been no direct evidence to incriminate immune complexes as the factor responsible for cerebral malaria. There is, however, indirect evidence suggesting that their role in the pathogenesis of cerebral malaria is as follows:

2.2.1 Cerebral malaria patients had more circulating immune complexes as detected by Ciq-binding assay than did NCM patients.[27]

2.2.2 Raised plasma level of C3d and decreased serum level of C3 and C4 in CM patients.[27]

2.2.3 Cerebral involvement is rare in malaria patients with protein calorie malnutrition (PCM),[28] and complement is low in malnourished children.[29] It could be possible that, in PCM patients, the immune complexes formed could not bring about a cascade reaction in the presence of a low complement level so as to produce the symptoms of cerebral malaria.

In an attempt to demonstrate the presence of immune complexes in patients who died of cerebral malaria, an immunoperoxidase staining of brain tissue for IgG, IgM and IgA was made, but no positive reaction could be demonstrated (M.J. Warrell, Department of Clinical Tropical Medicine and Hospital for Tropical Diseases, Faculty of Tropical Medicine, personal communication).

In our study, immune complexes were also determined (S. Khusmith, Faculty of Tropical Medicine, Bangkok, unpublished observation). Our results using Ciq-binding assay confirmed the findings of Adam[26] that immune complexes were demonstrated in CM patients more frequently than in NCM patients. However, it remains to be seen whether such complexes have any malaria antigen and whether they have any role in the pathogenesis of cerebral malaria.

2.3 Defects in non-specific immunity

There have been no reports regarding the study of changes in non-specific factors involving host defence against malaria in cerebral malaria patients. Factors that should be studied include the response of leukocytes to chemotactic stimuli, the phagocytic and microbicidal functions of peripheral blood leukocytes, interferons, natural killer cell and K-cell functions.

In conclusion, it appears that several factors could be involved in the development of cerebral malaria, and it is not known whether the basis of pathogenic mechanism of cerebral malaria in different cerebral malaria patients are the same or different. There is more to be learned from an experiment of this nature; a thorough understanding of the pathogenesis of cerebral malaria will help in the management of this severe form of malaria.

Savanat Tharavanij, M.D., Ph.D.
Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

REFERENCES

1. Daroff RB, Deller JJ, Kastl AJ, Blocker W. J Am Med Assoc 1967; 202:679-82.
2. Harinasuta T, Dixon KE, Warrell DA, Doberstyn EB. Recent advances in malaria with special reference to Southeast Asia. Southeast Asian J Trop Med Pub Hlth 1982; 13:1-34.
3. Rigdon RH, Fletcher DE. Lesions in the brain associated with malaria: pathologic study on man and on experimental animals. Arch Neurol Psychiatry 1945; 53:191-8.
4. Arieti S. Histopathologic changes in cerebral malaria and their relation to psychotic sequels. Arch Neurol Psychiatry 1946; 56:79-104.
5. Toro G, Roman G. Cerebral malaria. Arch Neurol 1978; 35:271-5.
6. Looareesuwan S, Warrell DA, White NJ, et al. Do patients with cerebral malaria have cerebral edema? A computed tomography study. Lancet 1983; 1:434-7.
7. Edington GM. Carebral malaria in the Gold Coast, Africa: four autopsy reports. Ann Trop Med Parasit 1954; 48:300-6.
8. Maegraith B, Fletcher A. The pathogenesis of mammalian malaria. Adv Parasit 1972; 10:49-75.
9. Udeinya IJ, Schmidt JA, Aikawa M, Miller LH, Green I. Falciparum malaria-infected erythrocytes specifically bind to cultured human endothelial cells. Science 1981; 213:555-7.
10. Clark IA. Does endotoxin cause both the disease and parasite death in acute malaria and babesiosis? Lancet 1978; 2:75-7.

11. Tubbs H. Endotoxin in human and murine malaria. Trans Roy Soc Trop Med Hyg 1980; 74:121-3.
12. Usawattanakul W, Tharavanij S, Warrell DA, et al. Factors contributing to the development of cerebral malaria. II. Endotoxin (manuscript under preparation). 1984.
13. Paswell JH, Dayer JM, Merler E. Increased prostaglandin production by human monocytes after membrane receptor activation. J Immunol 1979; 123:115-20.
14. Kontos HA, Wei EP, Povlishock JT, Dietrich WD, Magiera CJ, Ellis EJ. Cerebral arteriolar damage by arachidonic acid and prostaglandin G2. Science, 1980; 209: 1242-5.
15. Egan RW, Paxton J, Kuehl FA Jr. Mechanism for irreversible self-deactivation of prostaglandin synthetase. J Biol Chem 1976; 251:7329-35.
16. World Health Organisation. Development in malaria immunology. Tech Rep Ser No. 579, 1975:45.
17. Tharavanij S, Warrell MJ, Tantivanich S, et al. Factors contributing to the development of cerebral malaria. I. Humoral immune responses. Am J Trop Med Hyg 1984; 33:1-11.
18. Cohen S, Butcher GA, Crandall RB. Action of malaria antibody *in vitro*. Nature 1969; 223:368-70.
19a. Perrin LH, Dayal R, Rieder H. Characterisation of antigens from erythrocyte stages of *Plasmodium falciparum* reacting with human immune sera. Trans Roy Soc Trop Med Hyg 1981; 75:163-5.
19b. Perrin LH, Dayal R. Immunity to asexual erythrocytic stages of *Plasmodium falciparum:* role of defined antigens in the humoral response. Immunol Rev 1982; 61:245-69.
20. Schmidt JA, Udeinya IJ, Leech JH, et al. *Plasmodium falciparum* malaria. An amelanotic melanoma cell line bears receptors for the knob ligand on infected erythrocytes. J Clin Invest 1982; 70:379-86.
21. Chulay JD, Aikawa M, Diggs C, Haynes JD. Inhibitory effects of immune monkey serum on synchronised *Plasmodium falciparum* culture. Am J Trop Med Hyg 1981; 30:12-19.
22. Tharavanij S, Patarapotikul J, Supavan V, Schelp FP, Warrell MJ. Serum alpha-1 antichymotrypsin is a possible growth inhibitor of *Plasmodium falciparum*. Southeast Asian J Trop Med Pub Hlth 1983; 14: 349-52.
23. Brown GV, Anders RF, Mitchell GF, Heywood PF. Target antigens of purified human immunoglobulins which inhibit growth of *Plasmodium falciparum in vitro*. Nature 1982; 297:591-3.
24. Thammapalerd N, Tharavanij S, Patarapotikul J, Warrell MJ. Radioimmunoprecipitation of sera from acute malaria and cerebral malaria patients. (Manuscript under preparation). 1984.
25. Tapchaisri P, Asavanich A, Limsuwan S, Tharavanij S, Harinsuta KT. Antibodies against malaria sporozoites in patients with cerebral and non-cerebral malaria. (Manuscript under preparation). 1984.
26. Brasseur PH, Agrapart M, Ballet JJ, Druihle P, Warrell MJ, Tharavanij S. Impaired cell-mediated immunity in *Plasmodium falciparum*-infected patients with high parasitaemia and cerebral malaria. Clin Immunol Immunopathol 1983; 27:29-38.
27. Adam C, Geniteau M, Gougerot-Pocidalo M, et al. Cryoglobulins, circulating immune complexes, and complement activation in cerebral malaria. Infect Immun 1981; 31:530-5.
28. Edington GM. Pathology of malaria in Africa. Brit Med J 1967; 1:715-8.
29. Sirisinha S, Suskind R, Edelman R, Charuputana C, Olson RE. Complement and C3 proactivator levels in children with protein calorie malnutrition and the effect of dietary treatment. Lancet 1973; 1:1016-20.