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With 3 figures in the text

Host immune response and pathological expression in malaria: possible implications for malaria vaccines

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INTRODUCTION

Recent progress in parasite immunobiology has led to the identification of several plasmodial antigens representing the target of the protective antibody response of the infected host. As a consequence, some of these antigens have been envisaged as potential malaria vaccines in man. However, in spite of these achievements, the fine mechanisms which lead to the development of a state of partial protective immunity or to the triggering of immunopathology during malaria infection are not yet fully understood. Thus, it may be appropriate to evaluate the relative importance of individual host immune responsiveness to parasite epitopes involved in the induction of immunity, or of some immunologically mediated adverse reactions such as glomerulonephritis, anaemia, thrombocytopenia, and cerebral syndrome.

We have addressed this question using two differing approaches. First, the role of the genetic background of the host in regulating the intensity of the immune response against the repetitive epitope of *Plasmodium falciparum* circumsporozoite (CS) protein was studied. Second, the involvement of immune mechanisms in some complications of malaria, such as cerebral manifestations and thrombocytopenia, were investigated, with particular attention to the role played by certain T cell subsets.

HOST IMMUNE RESPONSE TO PLASMODIUM EPITOPES: CAN GENETIC RESTRICTION LIMIT THE EFFECTIVENESS OF FUTURE MALARIA VACCINES?

It was shown in experimental animal models and in human volunteers that inactivated malaria parasites were able to confer immune protection against infectious challenges (reviewed by Cochrane, Nussenzweig & Nardin, 1980). The production of monoclonal antibodies directed against plasmodial antigens and the progress achieved in molecular biology led to the characterization of several antigens able to confer some degree of immune protection against malaria infection (reviewed by Drager-Dayal & Lambert, 1986).

One of the most extensively studied malaria antigens is the circumsporozoite (CS) protein. Individuals living in malaria-endemic areas produce antibodies against these proteins after natural malaria infections and the anti-sporozoite antibody titres increase as a function of age: children under 2 years of age are mostly negative whereas the great majority of adults over 40 years are positive (Nardin *et al.* 1979; Tapchaisri *et al.* 1983; Zavala, Tam, Nussenzweig & Nussenzweig; 1985*a*; Del Giudice *et al.* 1986*a*, *b*).

The CS proteins consist of an amino acid sequence tandemly repeated several times and flanked by unrepeated sequences (reviewed by Nussenzweig & Nussenzweig, 1985). Most monoclonal antibodies so far produced against sporozoites and most anti-sporozoite antibodies induced after natural malaria infections bind specifically to the

repeated amino acid sequence (Zavala, Cochrane, Nardin, Nussenzweig & Nussenzweig, 1983).

The repetitive epitope of *P. falciparum* CS protein consists of 4 amino acids (Asn–Ala–Asn–Pro = NANP) repeated 37 times (Dame *et al.* 1984; Enea *et al.* 1984). $(NANP)_n$ sequences have been now produced by both chemical synthesis (Zavala *et al.* 1985*b*; Ballou *et al.* 1985) and by recombinant DNA technology (Young *et al.* 1985). It has been shown that 3 (NANP) repeats represent the minimal size of the molecule able to be recognized efficiently by specific antibodies (Zavala *et al.* 1985*b*) and to prime specifically murine T cells *in vivo* (Togna *et al.* 1986). $(NANP)_n$ peptides have been successfully employed for the detection of anti-*P. falciparum* sporozoite antibodies by means of enzyme-linked immunosorbent assay (Del Giudice *et al.* 1986*a*; Hoffman *et al.* 1986) or radio-immunometric assay (Zavala, Tam & Masuda, 1986). A large $(NANP)_{40}$ synthetic peptide was used as coating antigen for ELISA in a longitudinal study carried out in a rural community in Tanzania (Del Giudice *et al.* 1986*b*). It was found that, at 10 years of age, about half of the children who have been heavily exposed to malaria infections did *not* develop anti-NANP antibodies. Furthermore, considerable differences in antibody levels against NANP were observed in children living in different households but exposed to the same epidemiological conditions, although their antibody titres to asexual blood-stage antigens were similar. These data suggested that the genetic background may be of particular importance for the regulation of anti-sporozoite responses. $(NANP)_n$ synthetic peptides conjugated to carrier proteins and large recombinant peptide carrying an amino acid sequence of *E. coli.* were shown to be highly immunogenic in mice and rabbits: the antibodies raised against them recognized specifically *P. falciparum* sporozoites and inhibited the penetration of sporozoites into cultured hepatoma and hepatic cells and their maturation into exo-erythrocytic forms (Mazier *et al.* 1986).

The immunogenicity of $(NANP)_n$ sequences could be directly studied in our laboratories, both at T and B cell levels, using a novel large synthetic peptide consisting of 40 (NANP) repeats, $(NANP)_{40}$, without any carrier. C57BL/6 mice (H-2^b) responded strongly to $(NANP)_{40}$, producing high titres of IgG and IgM antibodies which also recognized specifically extracts of *Anopheles stephensi* mosquitoes infected with *P. falciparum* sporozoites. Congenic mice bearing the immunoglobulin allotype Ig^e responded in the same manner, as well as the (C57BL/6 × BALB/c)F1 hybrid mice. However, neither athymic C57BL/6 *nu/nu* nor congenic C57BL/6.H-2^k mice produced anti-$(NANP)_{40}$ antibodies (Del Giudice *et al.* 1986*c*). These results suggested that the production of antibodies to carrier-free $(NANP)_n$ peptides (i) T cell dependent, (ii) not dependent on the immunoglobulin allotype of mice and (iii) possibly linked to particular haplotypes of the murine major histocompatibility complex (MHC) H-2.

In addition, 14 mouse strains bearing 9 different H-2 haplotypes were immunized with $(NANP)_{40}$ without carrier but, surprisingly, only H-2^b mice (C57BL/6, C57BL/10 and BXSB) were able to mount a strong antibody response against the peptide, all non-H-2^b mouse strains tested being completely unresponsive (Fig. 1). The anti-(NANP) antibody response was shown to be strictly linked to the presence of the *b* allele in the I–A region of the H-2 gene complex (Del Giudice *et al.* 1986*c*). In fact, only I–A^b recombinant B10.A(5R) mice produced such antibodies. Furthermore, the antibody response against $(NANP)_{40}$ was extremely weak in B6CH-2^{bm12} mutant mice, which carry a gene conversion leading to the substitution of 3 amino acids of the beta 1 domain of the I–A^b beta chain (McIntyre & Seidman, 1984). These findings were confirmed by experiments employing (NANP)-specific T cell clones derived from lymph node cells of C57BL/6

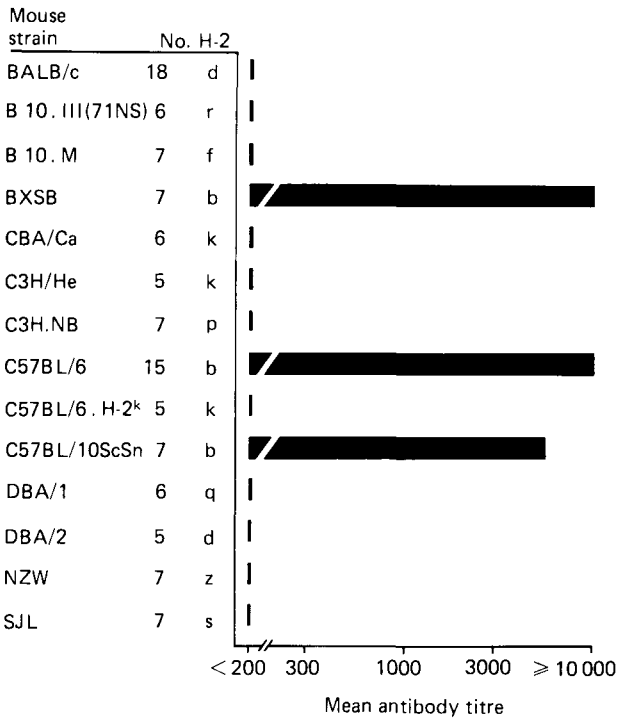

Fig. 1. IgG antibody response against $(NANP)_{40}$ in 14 mouse strains bearing 9 different H-2 haplotypes. Mice were immunized at the base of the tail with 50 μl of $(NANP)_{40}$ (20 μg) in Complete Freund's Adjuvant. Four weeks later the mice were boosted in the same way with the peptide in Incomplete Freund's Adjuvant. Sera were taken 7 days later and tested individually by ELISA, employing $(NANP)_{40}$ at a concentration of 1 μg/ml for coating the plates. Solid bars represent the geometrical mean of the titres obtained in each group. Titres of less than 1:200 were considered to be negative.

mice immunized with carrier-free $(NANP)_{40}$ (Togna *et al.* 1986). These L3T4$^+$ T cell clones proliferated only in the presence of irradiated spleen cells from C57BL/6 or B10.A(5R) mice as antigen presenting cells (APCs), not in the presence of APCs from B10.A(4R) nor from B6CH-2^{bm12} mice. T cell clone proliferation was specifically inhibited by adding an anti-I–A^b monoclonal antibody to the cultures. A similar genetic restriction of the immune response to NANP, at the T cell level, was recently reported by Good *et al.* (1986). The inability to respond to $(NANP)_{40}$ could be overcome in non-H-2^b mice when they were immunized with the peptide conjugated to a carrier protein (Del Giudice *et al.* 1986*c*).

Thus, the murine antibody response to $(NANP)_n$ is genetically restricted and strictly linked to the presence of a particular allele (*b*) in the I–A region of the H-2 complex. This exceptional restriction is surprising since, usually, mice of more than one H-2 haplotype are able to respond to a given epitope (reviewed by Schwartz, 1986). One can postulate that an analogous genetic control of the antibody response to $(NANP)_n$ peptides could also exist in man, and may limit the effectiveness of a vaccination with the $(NANP)_n$ peptides. On the other hand, such genetic control could also exist for the antibody response to whole *P. falciparum* sporozoites. This could also explain the observation that a significant percentage of young subjects living in malaria endemic areas do not develop anti-NANP antibodies (Zavala *et al.* 1985*a*; Del Giudice *et al.*

1986*a*, *b*). Further studies are required in order to determine the actual role played by MHC gene products in man in controlling the ability of different individuals to respond to sporozoites and/or to sporozoite peptides.

It was shown that the H-2 restriction existing towards the immunodominant repetitive epitope of *P. falciparum* CS protein is overcome when a peptide–carrier protein conjugate is employed as an immunogen. In these conditions, there is an expansion of B cells specific for the repetitive epitope, but often no priming of NANP-specific T cells. Thus, individuals vaccinated with such conjugates may lack sporozoite-specific T cells and, in endemic countries, would not benefit entirely from the boosting effect provided by natural sporozoite inoculations.

CAN MALARIA VACCINES ENHANCE IMMUNOPATHOLOGY? EVIDENCE FOR IMMUNOLOGICAL MECHANISMS IN SOME COMPLICATIONS OF EXPERIMENTAL MALARIA

In view of the presently available immuno-epidemiological data, it is conceivable that any future malaria vaccines would only confer partial immunity against plasmodium infection and one should expect a lack of vaccine efficiency in at least a small proportion of immunized children. Therefore, it may be essential to evaluate the relative risk of enhancing immunopathology by inducing an immune response towards selected plasmodial epitopes before natural exposure to malaria infection. This concern is largely based on experimental evidence suggesting that some major complications of malaria could partly result from the immune response against parasite antigens. This is particularly the case for glomerulonephritis, anaemia, thrombocytopenia, cerebral malaria and tropical splenomegaly syndrome.

The pattern of individual immune responses, particularly at the T cell level, and the relative involvement of certain identified plasmodium antigens have been studied in our laboratory in relation to the development of some of these pathological reactions during murine malaria.

Cerebral malaria

Cerebral symptoms accompanying acute falciparum malaria in young children and in non-immune adults represent severe complications with a frequent fatal outcome. The most common autopsy findings are vascular congestion and plugging of blood vessels with heavily parasitized erythrocytes, petechial haemorrhages and reactive gliosis (Polder, Jerusalem & Eling, 1983). The neurons usually show no obvious lesions besides signs of anoxia associated with localized cerebral oedema in the perivascular areas. Brain oedema is a common finding at autopsy (Aikawa, Suzuki & Gutierrez, 1980). However, recent studies have shown by computed tomography that cerebral oedema may occur but is not a consistent feature of severe cerebral malaria (Looareasuwan *et al.* 1983). *In vivo* damage to the endothelial cell, with resulting alteration of capillary permeability has been reported in patients with cerebral malaria (Areekul, Kasemsuth & Kanakakorn, 1984) and increased permeability of the choroid plexus has been shown in rodent malaria (Depierreux, Hochmann, Herrera & Lambert, 1986). The pathogenesis of these charges remains unknown, although various hypotheses have been proposed: endothelial lesions (Knisely, 1961; Wash, 1979), with attachment of monocytes to the endothelium (Rest & Wright, 1979; Rest, 1982); sequestration of parasitized red blood cells in capillaries (Yoeli & Hargreaves, 1974; McPherson *et al.*

1985); allergic reaction of the central nervous system to antigenic challenge (Toro & Roman, 1978); T-cell-mediated cellular immune reaction (Wright, Masembe & Bazira, 1971; Finley, Mackey & Lambert, 1982) or T-cell-dependent humoral reaction involving circulating immune complexes (Contreras, June, Perrin & Lambert, 1980; Rest & Wright, 1979; Adam *et al.* 1981) and the activation of the complement cascade.

In man, indirect evidence of a T cell involvement in neurological complications is provided by the observation of lower frequency of cerebral malaria in malnourished children (Edington, 1967). Thymic atrophy (Watts, 1969; McFarlane, 1971) and functional impairment of cell-mediated responses (McMurray, 1984) have been documented. Immune mechanisms, and particularly T cell mediated immunity have been implicated in the development of experimental cerebral malaria, since neurological complications are less severe in neonatally thymectomized hamsters (Wright *et al.* 1971) and absent in athymic nude BALB/c mice (Finley *et al.* 1982).

In our laboratory, a mouse model of cerebral malaria, after infection with *Plasmodium berghei* asexual blood stages, was analysed. In this experimental model, a cumulative mortality of about 90% between day 7 and day 15 of infection was observed in CBA mice, which appeared to be genetically susceptible to the development of neurological lesions. A particularly important feature of this model is that cerebral signs occurred when anaemia was moderate and the parasitaemia relatively low. In addition, several clinical and histopathological parameters were found to be similar to those observed in patients with cerebral malaria (Jerusalem *et al.* 1983; Grau *et al.* 1986). Three lines of evidence indicate that helper T lymphocytes play a significant role in the development of murine cerebral malaria (Grau *et al.* 1986).

First, it was demonstrated that the *in vivo* depletion in L3T4$^+$ helper/inducer T cells induced by treatment of *P. berghei*-infected mice with an IgG2b monoclonal antibody (MAb) directed against the L3T4 molecule, completely abrogated the occurrence of cerebral malaria, although there was no modification of the infection itself (Grau *et al.* 1986). No protective effect was seen after treatment of infected mice with a MAb of the same isotype directed against Ly.2$^+$ T cell subset. The effectiveness of this treatment by anti-L3T4 MAb in infected mice was demonstrated both phenotypically (depletion of the corresponding T cell subset) and functionally (inhibition of the IgG antibody response to a T-dependent antigen, tetanus toxoid).

Second, experiments were conducted in adult-thymectomized, irradiated and bone marrow-reconstituted (AT × BM) CBA mice which appeared to be completely resistant to the development of neurological lesions upon infection with *P. berghei*. These results confirmed and extended the results obtained using athymic *nu/nu* mice (Finley *et al.* 1982) which suggested a role for T cells in the development of cerebral malaria. T cells carrying the L3T4 phenotype were shown to be particularly involved in this syndrome since reconstitution of AT × BM CBA mice with normal L3T4$^+$ T cells rendered these mice fully susceptible to the development of neurological complications. In contrast, AT × BM mice reconstituted with the Ly.2$^+$ T cell subset did not die acutely with neurological signs but later developed severe anaemia and overwhelming parasitaemia (Grau *et al.* 1986).

Third, there was an exacerbation of neurological signs and earlier mortality observed after transfer of L3T4$^+$ Ly.2$^-$ T cells from mice with cerebral malaria into infected euthymic mice. This observation supported the hypothesis of an involvement of these cells in the pathogenesis of cerebral malaria.

Humoral parameters of the immune reponse were also analysed in *P. berghei*-infected CBA mice (Grau *et al.* 1986). As previously suggested (Rosenberg, 1978; Finley *et al.*

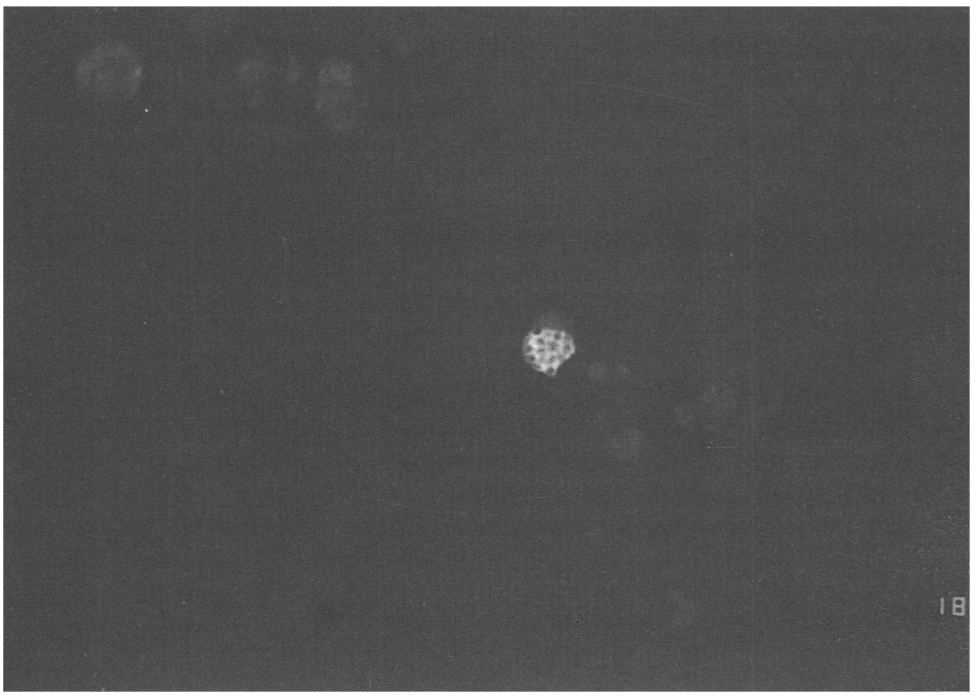

Fig. 2. Detection, by indirect immunofluorescence, of specific antibodies directed against segmented schizonts in the serum of a *Plasmodium berghei*-infected CBA mouse developing cerebral malaria 9 days after infection.

Table 1. *Effects of T-cell depletion or reconstitution on anti-*Plasmodium *antibody response during infection.*

	Anti-*Plasmodium* antibodies		
		Specificity	
Mice	IFA titre*	RF, TZ	Seg. SZ
Euthymic	8173	+	+
Euthymic			
+ anti-L3T4 MAb	469	+	−
+ anti-Ly.2 MAb	729	+	+
AT × BM	420	+	−
AT × BM.4$^+$	3040	+	+
AT × BM.2$^+$	420	+	−

* Day 14.

1982) malaria-associated polyclonal B cell activation was found to be T cell dependent. Indeed, in infected mice treated with anti-L3T4 MAb, there was a marked inhibition of the increase in serum levels of IgG, IgM and circulating immune complexes (CIC) compared with untreated infected animals. The specific anti-plasmodium immune response was also studied, with particular attention to antibodies recognizing certain stages of parasite development (Fig. 2, Table 1). In fact, in malaria-infected mice treated with anti-L3T4 MAb, there was a significant decrease of the specific anti-plasmodium antibody response but this response was in no way completely suppressed. The quality

of the response appeared to be more specifically influenced by the treatment with anti-L3T4 MAb. Antibodies directed against mature stages of plasmodium (polysegmented schizonts) were consistently absent in the sera from those L3T4$^+$ T cell-depleted mice which were protected against cerebral malaria (Table 1). Moreover, anti-segmented schizont antibodies were found in only 1 of 15 sera from day 8-infected euthymic CBA mice without cerebral malaria. The absence of anti-segmented-schizont immune response in the anti-L3T4 treated mice might be of importance in the protecting effect of this treatment.

In addition, the hypergammaglobulinaemia and the increase in CIC levels, which were absent in AT × BM mice, were restored in AT × BM mice reconstituted with normal L3T4$^+$ T cells (AT × BM.4$^+$). Specific antibody levels indicated that AT × BM mice responded quite poorly to malaria antigens. Although this response was only moderately enhanced by the adoptive transfer of L3T4$^+$ T cells, it is noteworthy that this transfer led to the appearance of anti-segmented schizont antibodies, already detectable by day 10.

The data presented here indicate that experimental cerebral malaria is probably mediated by immune mechanisms. Indeed, it was shown that the development of murine cerebral malaria is not directly related to the degree of anaemia and parasitaemia, but rather appears as the expression of immunopathological reactions of the infected host. The importance of the functional integrity of lymphoid cells expressing the L3T4 phenotype (helper T cells) in the triggering of neurological complications was outlined in these studies. One should note that L3T4$^+$ T cells of different specificities for malaria antigens may have different effects on the infection and, particularly, on the cerebral complications.

Various functional relationships between L3T4$^+$ T cells and neurological lesions can be envisaged (Fig. 3). Firstly, the helper effect of L3T4$^+$ T cells for the specific anti-plasmodium antibody response may be of particular importance, since it was shown that antibodies of certain specificities (segmented schizont antigens) are consistently associated with the occurrence of cerebral malaria. This helper effect may also be exerted in a less specific manner: malaria-associated polyclonal B-cell activation appears indeed to be largely T cell dependent. Secondly, the activation of T cells in the presence of properly presented malarial antigens results in the release of various lymphokines such as interleukin 2, interleukin 3, colony-stimulating factor and γ-interferon. The production of γ-interferon was demonstrated *in vitro* by T cells in the presence of macrophages and parasitized erythrocytes (Ockenhouse, Schulman & Shear, 1984). This was associated with the release of reactive oxygen species, participation of which has been documented in acute vascular changes (Chan, Schmidley, Fishman & Longar, 1984) and suggested in the pathogenesis of cerebral malaria (Clark & Hunt, 1983; Clark, Hunt & Cowden, 1987). Tumor-necrosis factor (TNF) is another intermediate produced upon macrophage activation which is known to alter selectively endothelial cell functions (Nawroth *et al.* 1986). Thirdly, L3T4$^+$ T cells can also mediate delayed-type hypersensitivity (DTH). However, the existence of local DTH-like reactions in the cerebral compartment was not suggested by histological studies since there was no accumulation of lymphocytes at the site of brain lesions (Jerusalem *et al.* 1983; McPherson *et al.* 1985; Depierreux *et al.* 1986).

The relevance of these data to human pathology should be discussed. Indeed, it has been suggested that immune mechanisms are not involved in the pathogenesis of human cerebral malaria (see Warrell, this issue) because there was no evidence, at autopsy, of cellular infiltrates nor of visible endothelial damage in the brain (McPherson *et al.* 1985).

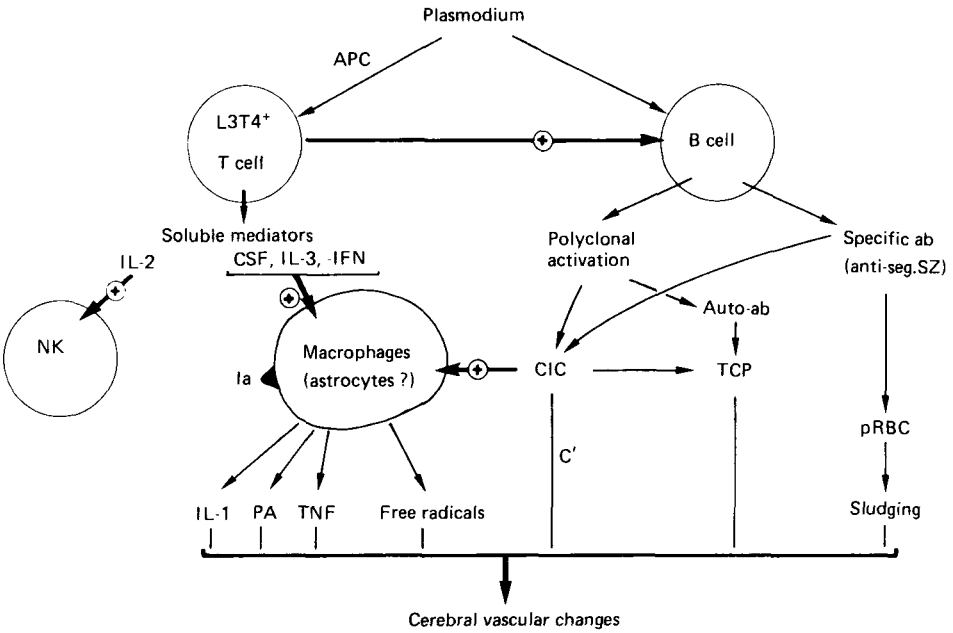

Fig. 3. Schematic representation of various mechanisms by which L3T4+ T cells can lead to the triggering of cerebral vascular changes (see text for discussion). APC, antigen presenting cell; CSF, colony-stimulating factor; seg. SZ, segmented schizonts; CIC, circulating immune complexes; TCP, thrombocytopenia; pRBC, parasitized red blood cells; PA, plasminogen activator; TNF, tumour necrosis factor.

In our experiments in mice, there were no lymphoid cell infiltrates in the brain and the vascular lesions were acute and localized to certain cerebral territories. Obviously, the sole classical morphological analysis does not allow us to draw conclusions regarding the existence of immunopathological mechanisms. However, since all these lesions were prevented in mice with an impaired immune response and appeared again after restoration of the L3T4+ T cell compartment, indirect mechanisms dependent on the specific or non-specific immune response to malaria are probably involved in the pathogenesis of the syndrome in mice. One cannot exclude the possibility that similar indirect mechanisms are also responsible for the cerebral syndrome in man.

EFFECTS OF CYCLOSPORIN A ON CEREBRAL MALARIA

Cyclosporin A (CsA) is an immunosuppressive agent capable of interfering at several levels with the immune response (Borel, Feurer, Magnee & Staehelin, 1977; Larsson, 1980; Bunjes, Hardt, Rollinghof & Wagner, 1981; Shevach, 1985). The T cell specificity for the immunosuppressive effects of CsA was suggested by the failure of CsA to suppress antibody response to bacterial lipopolysaccharide antigens *in vivo* in nude mice. Recent data suggest that the major site of action of CsA is the inhibition of mRNA transcription for the lymphokines interleukin 2 (IL-2), interleukin 3 (IL-3) and interferon-γ (IFN-γ) (Kronke *et al.* 1984; Elliott *et al.* 1984; Granelli-Piperno, Inaba & Steinman, 1984; Wiskocil *et al.* 1985). CsA inhibits lymphokine production in both helper and cytolytic cells, but does not directly inhibit or block proliferative responses of cytolytic cells in which proliferation can occur via an IL-2 independent pathway (Herold *et al.* 1986). Because of the powerful inhibitory effects of CsA on helper T cells and since these helper

T cells have been shown to play a major role in the pathogenesis of murine cerebral malaria, the potentially beneficial effects of CsA were evaluated in the murine model of *P. berghei* infection.

A direct toxic effect of CsA on malaria parasite has been documented (Thommen-Scott, 1981; Nickell, Scheibel & Cole, 1982). CsA has therefore been administered at different doses, especially at doses unable to exert any anti-parasite effect. It was shown that, in these conditions, CsA as well as some CsA analogues were able to protect fully more than 90 % of treated animals (Grau *et al.* manuscript submitted). As little as 1 mg CsA/kg body weight, given during 5 consecutive days once the infection had entered its patent phase, displayed a strong protective effect on cerebral complications without interfering at all with the progression of parasitaemia and without any detectable effect on humoral immune responses. Higher doses of CsA delayed the increase of parasitaemia and, as a consequence, cerebral malaria was delayed but in no way prevented as in mice treated with lower doses. The mechanisms by which low doses of CsA exert their protective effects on cerebral complications are not yet understood, but the *in vivo* evaluation of post-translational inhibition of mRNA for various lymphokines is under current investigation.

These results provide promising implications for treatment of malaria-infected patients, with or without severe complications. Preliminary results have indicated (J. F. Borel, personal communication) a beneficial effect of CsA in human patients with cerebral malaria. This observation tends to support the hypothesis of an immuno-pathogenesis of cerebral malaria.

MALARIA-ASSOCIATED THROMBOCYTOPENIA

Thrombocytopenia is a frequent complication in patients with severe malarial infection (Beale, Cormack & Oldrey, 1972; Butler *et al.* 1973; Horstmann, Dietrich, Bienzle & Rasche, 1981; Wilson, Neame & Kelton, 1982).

The mechanism of this thrombocytopenia remains poorly understood. The rapidity of onset and recovery as well as reduced platelet survival (Skudowitz *et al.* 1973; Horstmann *et al.* 1981) suggests that it is caused by increased platelet destruction. Disseminated intravascular coagulation (DIC) has been considered as a possible cause in some investigations (Dennis, Eichelberger, Inman & Conrad, 1967; Borochowitz, Crosley & Metz, 1970) but, in recent years, it has been recognized that thrombocytopenia is a very common and early sign of malaria infection, whereas DIC is distinctly uncommon (Beale *et al.* 1972; Srichaikul *et al.* 1975; Vreken & Cremer-Goote, 1978). Direct interactions between plasmodium and platelets have also been suggested. Parasites have been demonstrated inside blood platelets by electron microscopy in both human and experimental infections (Fajardo, 1973; Fajardo & Tallent, 1974; Fajardo, 1979), but the precise implications of this finding remain obscure. Immune mechanisms may also mediate thrombocytopenia and can be potentiated by the generalized reticulo-endothelial system (RES) hyperplasia found at autopsy and the increased RES functional activity during acute malaria (Sheagren, Tobie, Fox & Wolfe, 1970). Immune complexes which are present in the circulation of malaria-infected patients could play a role in the peripheral destruction of platelets as well as red blood cells (Contreras *et al.* 1980). Some evidence for such immune mechanisms has been provided by the quantification of platelet-associated IgG (PAIgG) in patients with acute malaria infection. Increased PAIgG levels are thought to be responsible for the rapid clearance

of circulating platelets by the RES and are currently considered to be a criterion for definition of immune thrombocytopenia (Kelton & Gibbons, 1982). Kelton, Keystone, Proctor & Neame (1980) reported increased levels of PAIgG in the majority of patients with malaria and thrombocytopenia. PAIgG returned to normal as the parasites were cleared from the circulation and the platelet counts returned to normal values. Recently, Kelton *et al.* (1983) demonstrated that, in patients with *P. falciparum* malaria and thrombocytopenia, IgG binds to platelet-associated malarial antigens through its Fab terminus, probably representing anti-platelet antibodies.

In our mouse model of infection with *P. berghei* asexual blood stages, thrombocytopenia develops as early as day 4 and its severity increases gradually until death.

Thrombocytopenia associated with *P. berghei* infection is likely to be due to an increased peripheral destruction rather than a central hypoproduction of platelets, since in infected mice, bone-marrow megakaryocytes were found in increased numbers and the survival time of ^{111}Indium-labelled platelets was reduced (Grau *et al.* manuscript submitted). The role of immune mechanisms was confirmed by the observation of increased amounts of IgG on platelets from infected animals. Elevated PAIgG correlated inversely with platelet counts. In addition, it was shown that serum from infected mice contains anti-platelet antibodies, as measured by an enzyme-linked immunosorbent assay (ELISA) using platelet monolayers as the solid phase (Grau *et al.* manuscript submitted). This assay was shown to be independent of the binding of circulating immune complexes through the Fc receptor. The functional *in vivo* importance of these serum anti-platelet antibodies was outlined by the fact that passive transfer of ultracentrifuged serum from *P. berghei*-infected, thrombocytopenic mice led to an immediate drop in platelet counts in syngeneic recipients (Grau *et al.* manuscript submitted).

The occurrence and the degree of *P. berghei*-induced thrombocytopenia was found to be dependent upon the T cell status of the host. Indeed, treatment of infected mice with an IgG2b MAb directed against the L3T4 surface antigen protected these mice against thrombocytopenia. In contrast, treatment with anti-Ly.2 MAb of the same isotype had no protective effect on thrombocytopenia. Similarly, there was no significant thrombocytopenia in AT × BM mice infected with *P. berghei*. The T cell dependency of *P. berghei*-induced thrombocytopenia was further demonstrated in experiments using AT × BM mice selectively reconstituted with normal L3T4$^+$ or Ly.2$^+$ T cell subpopulations. Indeed, the susceptibility to develop thrombocytopenia upon infection with *P. berghei* was restored only in AT × BM mice previously reconstituted with L3T4$^+$ T cells. More direct evidence of the role of L3T4 T cells in the development of *P. berghei*-induced thrombocytopenia was provided by another transfer. L3T4$^+$ Ly.2$^-$ T cells were isolated from *P. berghei*-infected mice undergoing cerebral malaria and severe thrombocytopenia. After elimination of contaminating parasitized erythrocytes, these cells were adoptively transferred to normal syngeneic recipients and were able to elicit a significant degree of thrombocytopenia.

Apart from antibody-mediated mechanisms, interactions between lymphoid cells and platelets have been described *in vitro* (Coeugniet, 1979; Gengozian & Rice, 1982) and might also play a role in the effect observed in such cell transfer experiments. Taken together, these results indicate that, in this experimental model, the thrombocytopenia which occurs during malaria infection displays all the parameters of an immunopathological complication. It is noteworthy that, in T cell-depleted mice, such as anti-L3T4, monoclonal antibody-treated or AT × BM mice, the protection against thrombocytopenia paralleled the protection against cerebral malaria. Thrombocytopenia may thus conceivably represent one of the multiple factors possibly involved in the triggering of

cerebral complications of malaria, by favouring the occurrence of brain haemorrhages (Fig. 3).

CONCLUSION AND SUMMARY

Until recently, the immune response to plasmodia has been considered without discriminating the relative importance of individual antigens or distinct epitopes in the induction of this response. It now appears that the general rules which regulate the operation of the immune system also apply to the immune responsiveness to single plasmodium epitopes. Thus the development of anti-parasite immunity, as well as the triggering of immunopathological manifestations, are probably strictly related to the variety of individual patterns of immune responsiveness encountered in populations exposed to the infection. These considerations are particularly relevant to the selection of antigens as potential candidates for malaria vaccination in order to achieve protecting efficiency without increasing the frequency of the well-known complications of acute malaria.

The immune response to *P. falciparum* CS protein repetitive epitope, NANP, is likely to be hampered by the requirement for an appropriate genetic background at the level of the MHC genes. This phenomenon was clearly shown in mice since only I–A^b mice were able to develop a proper T cell response and to produce anti-NANP antibodies after immunization with carrier-free peptide.

Preliminary epidemiological studies in man indicate that the repeated natural exposure to this epitope can induce an anti-NANP response but not in all individuals. Therefore, the relative importance of the genetic regulation of the immune response to defined plasmodium epitopes in man will have to be taken into account in order to avoid inefficient vaccination in a significant proportion of individuals at risk.

The analysis of experimental models of malaria in rodents underlines the potential risk of enhancing malaria-associated pathology by an inappropriate or a relatively inefficient immunization against selected plasmodium antigens. Indeed, T cells of the $L3T4^+$ subset seem to play a central role in the pathogenesis of murine cerebral malaria as well as in malaria-associated thrombocytopenia. The immune response to certain asexual blood-form antigens may be critical for the pathological expression of the disease. Obviously, potentially pathogenic responses are also genetically controlled and possible vaccine side-effects may be restricted to a limited segment of the population. However, in view of the large numbers of individuals exposed to malaria and likely to be vaccinated, the risk of enhancing immunopathology should not be neglected.

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