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The role of mitogenic factors in the pathogenesis of certain features of malarial infection

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Summary

The clinical and pathological features of malaria have been well recognized for a long time. Macroglobulinaemia accompanies malarial infections and these patients have increased susceptibility to secondary infection. They may also have splenic enlargement. However, how these changes are brought about is still not fully explained. For over a decade many researchers have looked into the possibility of a parasite-derived mitogen being partly responsible for some of these features. This paper appraises, in the light of evidence so far advanced, the role of mitogenic factors in the pathogenesis of hypergammaglobulinaemia, immunosuppression and splenomegaly associated with malarial infection. The nature of the stimulatory material in parasite extracts is also discussed.

Résumé

Depuis longtemps, l'on connaît les caractéristiques cliniques et pathologiques du paludisme. Une quantité élevée de globules dans le sérum accompagne l'infection paludéenne, et l'on a observé chez les patients traités une susceptibilité accrue à des infections secondaires. Il est possible qu'ils aient souffert d'une dilatation de la rate. On ne s'explique pas encore exactement, cependant, la façon dont ces changements surviennent. Depuis plus de dix ans maintenant, les chercheurs pensent qu'un mitogène provenant d'un parasite est à l'origine de certaines caractéristiques du paludisme. A la lumière de ce que nous savons déjà, nous évaluons dans cette communication le rôle des facteurs mitogéniques dans la pathogénie de l'excès de gammaglobuline dans le sérum, de la suppression du système d'immunisation et de la dilatation de la rate associées à l'infection paludéenne. Nous examinons également la nature des stimulatants dans des extraits de parasite.

Hypergammaglobulinaemia

Immunoglobulin M levels in serum increase rapidly during acute malarial infection [1]. Those exposed to recurrent attacks of malaria have high serum IgG and IgM levels [2,3]. Only a small proportion of the serum immunoglobulins in infected individuals contains antibody activity specific for the malaria parasite [4]; the remainder are antibodies with heterophile and autoimmune reactivity [5]. The diversity of the immune response was believed to suggest polyclonal B-cell activation, and the possibility of a malaria mitogen was therefore proposed [6,7]. Blast transformation of human peripheral blood lymphocytes [5,8–12] and rodent splenocytes [13–15] have been used to study the activity of extracts of *Plasmodium falciparum*- and *Plasmodium berghei*-infected red blood cells. The results ranged from the demonstration of antigen-specific stimulation of previously sensitized lymphocytes [8,12] to mitogen-like stimulation of both sensitized and non-sensitized lymphocytes [5,9,11,13].

A more direct evidence for lymphocyte (B cell) activation by a malaria 'mitogen' was presented by Freeman and Parish [14] who showed that a water-soluble extract of *P. berghei*-infected red blood cells greatly enhances IgM plaque formation by BALB/c mouse spleen cells *in vivo*. Also, it has been possible to induce hypergammaglobulinaemia in uninfected BALB/c mice with *P. berghei* culture supernatants [16]. The active factor in *P. falciparum* culture supernatants has been partially charac-

terized. It was found to be heat resistant, partially degraded by proteolytic enzymes (suggesting that it might be a glycoprotein) and in fractions with a molecular weight of about 200,000 daltons on gel filtration [5].

The putative mitogen from *P. falciparum* stimulated both B and T lymphocytes in experiments reported by Greenwood *et al.* [5] but Wyler *et al.* [11] obtained stimulatory response in T but not B cells. The purification method for B lymphocytes employed by Greenwood *et al.* [5] allowed some contamination with T cells. The response to the 'mitogen' was reduced by anti-theta serum [13], supporting the involvement of T lymphocytes. However, depletion of B and T cells occurs in the lymph nodes of mice infected with *P. berghei* [17]. Similar changes have been observed in the peripheral blood of children with acute *P. falciparum* malaria [18,19]. The results obtained by Greenwood *et al.* [19] indicate that T lymphocytes are relatively more depleted than B lymphocytes. T-lymphocyte depletion in acute malaria is not accompanied by significant impairment of cell-mediated immunity to skin-test recall antigens and sensitization with dinitrochlorobenzene [20]. However, the response of patients' lymphocytes to phytohaemagglutinin (PHA) was normal only on maximal stimulation [20] and was slightly reduced at suboptimal concentrations of the mitogen [21].

More recent evidence indicates that the proliferative response of lymphocytes from malaria patients to the T-cell mitogen La (leucoagglutinin from PHA) was significantly reduced, compared with that of controls [22]. With pokeweed mitogen (PWM), which stimulates T cells and induces a T cell-dependent activation of B cells, they found no difference in the proliferative response between patients and controls. However, Troye-Blomberg *et al.* [12] observed that *P. falciparum* extracts induce significant stimulation of DNA synthesis (peaking after 3–4 days of incubation) in malaria patients' lymphocytes. They noted that this response was specific for *P. falciparum* since it was not obtained with lymphocytes from healthy donors nor with those from patients with acute *P. vivax* or *P. ovale* malaria. In a malaria-endemic region in Upper Volta, Daniel-Ribeiro *et al.* [23] have found an abnormal prevalence of antinuclear antibodies in contrast to the normal frequency of other anti-tissue antibodies.

Immunosuppression

McGregor and Barr [24] have shown that children on malaria prophylaxis had a greater antibody response to tetanus toxoid than children who had not received prophylaxis. The fact that immunosuppression accompanies malarial infections is now well documented [20,25–28]. Investigations of malaria-induced immunosuppression in animals have shown that humoral immunity is affected more than cellular immunity [29]. Evidence for suppressor cell activity could not be found in malaria-infected mice [30, 31]. However, malaria results in a numerical increase [32] and activation [33] of macrophages in the spleen. Some of these macrophages may elaborate soluble mediators that inhibit lymphocyte proliferation [34]. There is evidence for disrupted accessory function of adherent splenic cells in malaria [35–37]. T cell-independent responses, like T cell-dependent responses, may require macrophage assistance [38]. Thus, immunosuppression may result from a defective accessory function of macrophages, and perhaps also from an activation of their suppressor potential [30,34]. Enhanced immunoglobulin synthesis [16] in the presence of macrophage-induced immunosuppression in malaria may be due to the fact that adherent cells that elaborate suppressor factors are only obtainable in the later stages of infection [34]. Mitogens were found to be unable to induce immunosuppression of the response to primary stimulus except when administered days before immunization [39,40], and it is possible that macrophage suppressor factors behave alike.

Other factors may also contribute to immunosuppression as mitogenic factors have been found associated with malarial infections [9,41]. B-cell mitogens have immunosuppressive potentiality [39,40]. Evidence has been presented to show that mitogenic extracts of *P. berghei* induce a depressed immune response to meningococcal polysaccharide vaccine in BALB/c mice [15].

Malarial splenomegaly

The spleen undergoes a dramatic increase in size in malarious animals and could reach up to fifteen times the size of normal spleen [42]. Untreated malarial infection may lead to the development of the tropical splenomegaly syn-

drome (TSS) [43] in some human patients. The pathogenesis of the splenic enlargement characteristic of TSS is still not fully explained. Ziegler [44] postulated that TSS results from prolonged stimulation of the reticuloendothelial system (RES) by circulating macromolecular immune complexes. Indirect evidence for immune complex formation and deposition in the RES has been presented [44–46] but correlation between serum immune complex level and splenic size could not be found [46]. Also, an attempt to induce splenomegaly in mice by injection of sera from TSS patients, which contain high levels of IgM and immune complexes, failed [47].

The production of mitogenic factors is characteristic of malarial infection [9,41]. Freeman and Parish [14] found that the numbers of plaque-forming cells were ten to forty times elevated in the spleens of uninfected BALB/c mice, injected with supernatants of lysates of *P. berghei*-infected red blood cells, when compared with mice injected with supernatants of uninfected red blood cells. Therefore, the involvement of a malaria 'mitogen' in the pathogenesis of the splenomegaly of malarial infection may be a rewarding hypothesis. Available evidence [48] failed to prove this hypothesis.

Discussion

The observation of depleted T-cell number [18,19,22] and enhanced immunoglobulin synthesis [1,16] in acute malaria suggests that there may be a selective depletion or inactivation of a subpopulation of T lymphocytes in this disease. These may be suppressor T cells, as patients with malaria have been observed to have a loss of functional Con A inducible T suppressor cells [49]. However, Troye-Blomberg *et al.* [22] observed a relatively elevated level of $T8^+$ T cells (expressed as percentage of total $T3^+$ cells) in *P. falciparum* patients. Also, Troye-Blomberg *et al.* [12] noted significant stimulation of DNA synthesis in patients' lymphocytes in response to parasite extracts and the responding cells may be mainly $T8^+$ suppressor T cells. However, these cells may be defective functionally. In a study of the relationship between H-2 expression and the induction of T cell-dependent immunity to plasmodia in mice, the T cells active in this system were shown to be 'contrasuppressor' cells [50]. These are a subset of Lyt-1^+ cells exerting their effects by inactivating T suppressor cells of Lyt-2^+ phenotype.

Lymphocyte activation by extracts derived from malaria parasites has been shown to be a T cell-dependent phenomenon [51] and the extract has been demonstrated to be a T-cell 'mitogen' [11]. T-cell mitogens can secondarily induce a functional B-cell response [52]. It is hereby suggested that a parasite-derived 'mitogen' is involved in the induction of hypergammaglobulinaemia in malarial infection; that the 'mitogen' operates through the activation of 'contrasuppressor' cells (or their functional equivalent in man), which inactivate suppressor T lymphocytes, thus resulting in excessive immunoglobulin production by B cells through unrestricted helper T-cell function. Wyler *et al.* [11] could not find increased numbers of spontaneously proliferating B cells in the circulation of patients with malaria but they examined only three patients. However, the possibility also exists that malaria preparations may be able to stimulate antibody secretion without inducing B-cell proliferation [11].

If 'contrasuppressor' cells (or their human equivalent) are active in malarial infections, suppressor lymphocytes will be hampered functionally and cannot be the explanation for immunosuppression associated with this condition. Tanabe *et al.* [31] and McBride and Micklem [30] failed to detect suppressor-cell activity in the response of infected mice to polyvinylpyrolidone and dextran, respectively. Immunosuppression in malaria seems associated with a temporary blockage of B-cell differentiation rather than destruction or permanent inactivation of responsive clones [30]. Thus, stimulation of antigen-sensitive cells by mitogenic factors may lead to emergence of a cell population that has temporarily lost its ability to react with antigen especially as the proliferative potential of B-cell clones is limited [53].

A possible mechanism of malaria 'mitogen' involvement in the production of splenomegaly may be through the polyclonal stimulation and consequent proliferation of splenocytes [48]. However, it is not everybody with malaria who develops splenomegaly. Why some develop splenomegaly and others do not may be due to genetic reasons as B-cell activity is regulated by the T-cell system. The efficient induction of a

specific T-cell response requires that the foreign antigen is presented to the T cells in the context of the major histocompatibility antigens (MHC-antigens) [50]. Meanwhile, there is no evidence of an excessive response to the polyclonal B-cell activation of malaria in subjects with TSS [50]. The fact that spleen cell proliferation may be induced in the presence of concomitant immunosuppression in malaria [14] may be explained by the fact that splenocytes may have been activated before macrophage suppressor factors were generated. Mitogens fail to induce suppression of the primary immune response except when administered before immunization [39,40]; it is possible that macrophage suppressor factors behave similarly.

The issue can be raised whether the stimulatory material in malaria parasite extracts is actually a mitogen. A close look at the literature and careful attention to results obtained indicate that the active factor demonstrates antigen specificity. If they exhibit mitogenic properties at all, this is not likely to be significant. The points that have been put forward for a mitogenic nature of the active factor in malaria include: (i) its ability to stimulate non-immune lymphocytes [5,9,14,15] and cord blood lymphocytes [5,8]; and (ii) the observation that a large number of autoantibodies is involved in malaria-induced auto-immunity [54–60].

Weinbaum *et al.* [13] have obtained results consistent with the possibility that the response by normal spleen cells to malaria extracts represents primary *in-vitro* sensitization, as this response exhibited delayed kinetics and reduced magnitude compared with the response by immune cells. The intense activity of the reticuloendothelial system during malarial infection [4] may result from stimulation by highly potent antigens from the parasite. This high potency of antigenic materials from plasmodia may be responsible for the response of cord blood lymphocytes, as evidence (i) against a functional immaturity, and (ii) for a lack of adequate triggering surface receptors on cord B lymphocytes, has been presented [61]. Also, the human foetus is able to respond immunologically to intrauterine infections [62–65]. While auto-antibodies directed against a number of auto-antigens have been described in malaria, Daniel-Ribeiro *et al.* [23] have found an abnormal frequency of only anti-nuclear antibodies (and not of 13 other auto-antibodies) in sera of 173 individuals living in a malaria-endemic region in Upper Volta. This selective increase in the frequency of one auto-antibody cannot result from a non-specific polyclonal mitogenic activation [23]. However, Phanuphak *et al.* [66] reported results associating acute malarial infection with high incidence of auto-antibodies to both nuclear and smooth muscle antigens. Another factor that may operate in the pathogenesis of auto-immunity could be repeated stimulation of the reticuloendothelial system with parasite antigens cross-reactive with normal tissue antigens [67].

Although there is evidence to show that only a fraction (6–11%) of the high serum immunoglobulin levels in malaria is specific malaria antibody [4], there seems to be no report indicating what proportion of the total immunoglobulin produced in response to malarial infection this fraction represents [68]. The results of Troye-Blomberg *et al.* [22] show that classical mitogens La and PWM induce proliferative responses in lymphocytes obtained from malaria-infected and uninfected individuals from Sweden — a malaria non-endemic area. Their observation [12] that *P. falciparum* extracts induced significant stimulation of DNA synthesis in patients' lymphocytes but not in those from the same group of healthy controls (as those studied with La and PWM) argues against a mitogenic nature for the active factor in malaria parasite extracts. Also, lymphocytes from patients with acute *P. vivax* or *P. ovale* malaria did not respond to stimulation with the same *P. falciparum* extract. The delayed proliferative responses (days 5–6) of lymphocytes from patients and healthy controls, obtained by Troye-Blomberg *et al.* [12] with *Plasmodium* extracts, were not significantly different from those obtained with control red blood cell extracts. This suggests that their parasite preparations do not contain mitogenic substances except if normal human erythrocytes elaborate mitogenic factors as they have suggested.

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