Altered Expression of Human Monocyte Fc Receptors in Plasmodium falciparum Malaria

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The state of activation of human peripheral blood monocytes was examined by using a rosette assay that detects changes in Fc receptor expression. Monocytes from patients with uncomplicated Plasmodium falciparum malaria showed a significant increase in the number of rosettes relative to healthy controls. In addition, the monocytes from these patients were tested for their ability to phagocytose Candida albicans, but this ability did not differ from that of normal individuals. Finally, the monocytes from patients with cerebral malaria were also tested for Fc receptor expression. In contrast to the results from uncomplicated cases, the activity of the monocytes from these patients was different from that of controls. We concluded that uncomplicated P. falciparum malaria caused an increase in monocyte Fc receptor expression which did not occur in cerebral malaria and that this difference in activation may be important in the pathogenesis of cerebral malaria.

It has long been established that the host response to plasmodia includes the production of enlarged populations of both peripheral blood monocytes and mature macrophages (28, 29). Splenomegaly and hepatomegaly are frequently observed in human malaria cases, and histological studies have demonstrated hyperplasia of the reticuloendothelial system (3). In rodent malaria, experiments have shown that both the spleen (20) and the liver (9) remove parasitized erythrocytes more rapidly from the circulation than they remove uninfected cells. Furthermore, macrophages from infected animals are more phagocytic for parasitized cells than are normal macrophages (8, 27), and this enhanced phagocytic activity depends on the presence of either opsonins (11, 30) or cytotoxic antibodies (10) and also on the degree of macrophage activation (27).

Although little is known about the nature of this macrophage activation, it seems reasonable to suppose that it includes increased expression of specific surface receptors for the Fc portion of immunoglobulin G (Fc receptors), since this would clearly facilitate the phagocytosis of antibody-coated targets. Moreover, increased Fc receptor expression has been observed in activated macrophages from adjuvant-stimulated animals and from experimentally induced inflammatory exudates (4, 21, 31). This finding suggests that changes in the expression of this receptor provide a useful indicator of macrophage activation.

Plasmodium falciparum infection is the most serious of the human plasmodia. Severe forms of the disease cause multiple organ dysfunction, and involvement of the central nervous system leading to coma (cerebral malaria) carries a mortality of 20% despite treatment (32). Cerebral malaria is characterized by a high parasite load but not necessarily a high parasitemia, because mature forms are sequestered in the microcirculation (17). In the present study, we decided to compare macrophage activation in uncomplicated P. falciparum malaria, in which the mononuclear phagocyte system might be activated and effectively aiding in defense against the parasite, with that in cerebral malaria, in which such defenses might have failed. Fc receptor expression by peripheral blood monocytes was chosen as an index of macrophage activation, and this paper reports the results.

MATERIALS AND METHODS

This study was conducted at Pra Pokkla Hospital, Chantaburi, Eastern Thailand, and ethical committee approval was granted by Mahidol University, Bangkok, Thailand.

Subjects. The groups of Thais in the study consisted of the following. (i) Twelve normal, healthy individuals aged between 16 and 47 years (mean age, 29 years). (ii) Thirteen inpatients with acute uncomplicated P. falciparum malaria who were receiving treatment with quinine. They were aged between 9 and 63 years (mean age, 24 years) with a median parasite count of 24,000 (range, 3,360 to 125,000) P. falciparum asexual forms per μl of blood. (iii) Five patients with cerebral malaria who were being treated with quinine. They were aged between 19 and 25 years (mean age, 22 years) and had a median parasite count of 3,400 (range, 1,320 to 62,500) P. falciparum asexual forms per μl of blood. Two of the five patients also had acute renal failure. Cerebral malaria was defined as the presence of asexual forms of P. falciparum in the blood of patients in a coma that could not be attributed to other causes (32). All samples were taken within 24 h of admission to the hospital.

Peripheral blood monocytes. From each subject, 10 ml of blood was obtained. It was defibrinated in nonwettatable plastic containers with a U-shaped glass rod. This process also removed platelets. Defibrinated blood was diluted 1 in 3 with phosphate-buffered saline, layered onto Ficoll-Paque (density, 1.078 g/ml) (Pharmacia Fine Chemicals, Uppsala, Sweden) and centrifuged for 40 min at 400 × g. The mononuclear cells at the interface were aspirated, washed, suspended in Hanks balanced salt solution (HBSS) containing 20% heat-inactivated fetal calf serum, then placed in tissue-culture chamber slides (Lab-Tek 4 chamber slides; Miles Laboratories, Slough, U.K.), and incubated for 1 h at 37°C. In these circumstances, the resultant adherent cell monolayers consist of more than 95% monocytes after being...
washed with HBSS to remove nonadherent cells (23). The monocytes of patients and normal donors were compared simultaneously in the following assays.

**Rosette assay for Fc receptor expression** (23). Antibody-sensitized sheep erythrocytes were prepared by incubating 2% washed erythrocytes with a range of concentrations of rabbit anti-sheep erythrocyte serum (Wellcome Research Laboratories, Beckenham, England) for 30 min at room temperature. The cells were then washed twice and suspended in HBSS at a concentration of 1%. Sensitized erythrocytes in 1-ml volumes were added to each monocyte monolayer and allowed to settle at room temperature for 1 h. The monolayers were then washed three times with HBSS and fixed with 0.5% glutaraldehyde for at least 1 h. The cells were stained with citrate-buffered Giemsa, the chambers were removed, and the slides were mounted with cover slips. Two hundred cells were counted per slide, and those cells bearing three or more erythrocytes were scored as rosettes and expressed as a percentage of the total monocyte population.

A range of suboptimal concentrations of antibody was used so that the binding of the reagent, visualized as rosette formation, could be expressed as a function of the dose of antibody. The resultant dose-response curves provided a sensitive reflection of differences between activated and nonactivated monocytes. At higher doses, however, all curves reached 100% rosette formation, indicating that all peripheral blood monocytes expressed detectable Fc receptors, whether activated or not. The rabbit reagent employed in the present study binds to the same receptor as human immunoglobulin G and is merely a convenient tool for assaying human monocyte Fc receptor expression (24).

**Phagocytosis assay.** Samples of washed *Candida albicans* (1 ml) suspended at suitable concentrations in HBSS with 5% fetal calf serum (Fig. 2) were incubated with the monocyte layers for 45 min at 37°C. The slides were then prepared, fixed, and stained as in the Fc receptor assay. Two hundred cells were counted on each slide, and the results were expressed as the average number of *C. albicans* ingested per monocyte.

**Statistical analysis.** The parasitc counts were not normally distributed, and the median value is therefore quoted rather than the mean. A normal distribution was assumed for the results obtained in the rosette assay for Fc receptor expression. In every experiment, monocytes from one or more patients with malaria were simultaneously compared with monocytes from a normal individual so that there would be a control for day-to-day variation in the assay. The data were therefore analyzed with a paired two-tailed *t* test, and the results are expressed as the mean ± the standard error of the mean.

**RESULTS**

Fc receptor expression in monocytes from patients with uncomplicated malaria was compared with that in normal individuals (Fig. 1). It is evident that there is a statistically significant increase in antibody-binding capacity of the monocytes from the infected individuals relative to the controls at all concentrations of sensitizing antiserum except the highest where each curve approached its maximum and, presumably, the cell sites were almost saturated. This change was clearly a function of increased Fc receptor activity, although no distinction could be made between greater receptor affinity and an increase in the number of available receptors.

The increased monocyte Fc receptor activity in uncomplicated malaria might reflect a more general cellular activation. We therefore decided to investigate the phagocytic activity of monocytes from these patients. Figure 2 shows the average number of *C. albicans* phagocytosed per cell at different concentrations of added *C. albicans*, and it can be seen that a dose-response curve was obtained. Monocytes were also tested from normal individuals, and there was no significant difference in phagocytic ability between the two groups.

Finally, the monocytes from the five patients with cerebral malaria were also tested for Fc receptor expression (Fig. 3). In contrast to the findings in uncomplicated malaria cases, the receptor activity in comatose patients did not differ from that of the healthy controls at any concentration of antibody tested.

**DISCUSSION**

The present study demonstrated that peripheral blood monocytes from patients with uncomplicated *P. falciparum* malaria show increased Fc receptor activity (Fig. 1). Enhanced Fc receptor expression has also been reported in monocytes from patients with malignancies (22, 23) and in patients with granulomatous diseases, namely, active pulmonary tuberculosis, sarcoidosis, and Crohn's disease (22, 25). These latter diseases are all chronic inflammatory conditions in which macrophage activation is expected.

The observed increase in the activity of monocyte Fc receptors in patients with uncomplicated malaria should facilitate antibody-dependent phagocytosis of parasites. It is already established that immune serum enhances the ability of normal monocytes to phagocytose *P. falciparum*-infected erythrocytes containing mature trophozoites and schizonts.
The pathophysiological mechanisms underlying cerebral malaria are unknown, but some researchers have suggested that it has an immunological basis. It is interesting therefore that in the present study, monocytes from patients with cerebral malaria did not show significantly increased Fc receptor expression (Fig. 3). This result contrasts with the enhanced Fc receptor activity observed in uncomplicated malaria. It is possible that the difference merely reflects the lower parasitemia observed in the peripheral venous blood of patients with cerebral malaria. However, such an explanation is unlikely because in cerebral malaria there is a marked dissociation between peripheral parasitemia and parasite load, owing to pooling of mature trophozoites and schizonts in the deep tissue capillaries, particularly in the brain (17). The parasite concentration in the blood may therefore grossly underestimate the total parasite numbers within the patient. In fact, increased circulating immune complexes have been demonstrated in cerebral malaria patients (2), and it seems most likely that the monocytes from comatose patients do have increased Fc receptor expression, as in uncomplicated malaria, but that the receptors are blocked by immune complexes. Such impairment of monocyte function could be important in the pathogenesis of cerebral malaria, and further studies should be carried out to examine this possibility.

In conclusion, although the exact role of the mononuclear phagocyte in resistance to malaria is unknown, this study shows that *P. falciparum* malaria can cause changes in monocyte Fc receptor activity which are likely to be of significance in the cellular immune response to the parasite.
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LITERATURE CITED


