IMMUNOLOGICAL EVALUATION OF CELL-MEDIATED AND HUMORAL IMMUNITY IN THAI PATIENTS WITH CEREBRAL AND NON-CEREBRAL *PLASMODIUM FALCIPARUM* MALARIA: I. Cutaneous Delayed Hypersensitivity, Blood Leukocytes and *In Vitro* Lymphocyte Responses

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INTRODUCTION

A limited number of studies on the impairment of cell-mediated immune functions are available in patients with falciparum malaria. Decreased numbers of blood T cells were observed in acute cases (MacDermott *et al.*, 1975 ; Wyler *et al.*, 1976; Greenwood *et al.*, 1977 ; Wells *et al.*, 1979). However, no major alteration of *in vitro* lymphocyte proliferative responses to lectins was found except in cerebral malaria cases (Williamson *et al.*, 1978 ; MacDermott *et al.*, 1980). Impairment of antibody responses to tetanus toxoid and other immunization antigens were found in *P. falciparum* infected African children (McGregor *et al.*, 1972 ; Greenwood *et al.*, 1972). In contrast, we have recently found that there was no detectable influence of low grade parasitaemia on cell-mediated and anti-tetanus antibody responses in a large group of African children (Monjour *et al.*, 1982; Ballet *et al.*, 1982). The present study was designed to determine whether *P. falciparum* infection exerted influences on non-specific cell-mediated immune responses in Thai patients including cerebral cases and high parasitaemia cases.

MATERIALS AND METHODS

Patients: Ninety-seven Thai patients with falciparum malaria admitted to Phra Pokklao Hospital, Chantaburi, East Thailand were studied, among whom 40 (25 males and 15 females) had cerebral malaria (CM). CM patients were defined as those having unrousable coma in the absence of other neurological disorders. Included in the CM group were 39 adults (mean age 27 years, range 15-60 years) and one 10-year-old child. Twenty six CM patients had had previous hospitalization with acute uncomplicated malaria ascertained by positive blood smear examination. Blood cultures for aerobic bacteria at the time of admission in CM patients were all negative. Among 57 patients without cerebral involvement (NCM), 52 were adults (27 males and 25 females) and 5 were children (2 males and 3 females). The mean age of adult NCM patients was 28 (range 15-68 years) and the age range in children was 9 to 12 years. None of the patients were clinically considered malnourished at the time of study.

In this report, the term "initial" applies to studies made within the first 24 hours of admission of the patient to the hospital.

The controls were healthy Thai individuals living in Chantaburi. Informed consents were obtained from the controls and from the patients or their legal representatives.

Cutaneous delayed reactions to phytohaemagglutinin and soluble antigens:

Skin tests were performed on the forearm skin by intracutaneous injection of 0.1 ml of phytohaemagglutinin (10 µg/ml PHA HA, Wellcome, Beckenham, England), tuberculin (10 iu/0.1 ml, Institut Pasteur, Paris), candidin (1 : 1000, Institut Pasteur) and streptokinase -streptodornase (40 IU/0.1 ml, Lederle, Madrid, Spain). Local skin induration and erythema were measured 48 hours later by the same investigator : and the mean size of induration of >5 mm was considered positive. Skin tests were performed at the day of admission and when negative response was recorded, skin tests were repeated 48 hours later.

Total leukocyte counts were performed in haemacytometers and differential counts were made from blood smears. Mononuclear cells were isolated from heparinized blood on Ficoll metrizoate gradients. On two occasions, spleen cells from CM patients obtained immediately post-mortem were studied. T cells were counted following rosette formation using aminoethyl-isothiouronium treated sheep erythrocytes (AET-E) (Pellegrino *et al.*, 1976). Membrane immunoglobulin bearing B cells detected with rhodamin-labelled $F(ab)'_2$ fragments of a rabbit IgG against human IgG (Nordic, Leyden, The Netherlands) (Preud' homme *et al.*, 1976).

Lymphocyte cultures were performed in 96-well microplates (0.2 × 10 cells in 0.2 ml per well) in RPMI 1640 medium supplemented with antibiotics and with heat inactivated human serum from AB blood donors. The lymphocytes were cultured for three days in the presence of phytohaemagglutinin (PHA-P, Difco, Detroit, USA; 1 : 600 of stock solution, poke-weed mitogen (PWM) Lederle 1 : 30 of stock solution) and concanavalin A (Con A, Pharmacia, Uppsala, Sweden, 10 µg/ml). Cells stimulated with CDD (100 IU/ml)and SKSD (250 µg/ml) were cultured for seven days. All cultures were performed in triplicate. Twelve hours before harvesting, two µci of 3H-thymidine (3HT) was added to each well. Thereafter the cells were harvested in an automatic cell harvester (MASH II) and the incorporated 3H-T was counted by scintillation spectrophometry. Statistical study was performed using the correlation coefficient r and X^2 test.

RESULTS

Cutaneous delayed reactions: Skin tests could be performed in 14 CM and 39 NCM patients. No reactions to PHA or other soluble antigens were observed in 15 patients (8/14 CM and 7/39 NCM patients). The 6 remaining CM patients reacted only to one out of 4 stimulants tested. In contrast 16 and 5 of the remaining 32 NCM patients responded to 2 and 3 stimulants respectively. The proportion of positive cutaneous responses to stimulants in the CM and in the NCM groups were significantly less than those in 15 adult controls ($p < 0.05$) in whom 2, 11, 0 and 2 were reactive to 4, 3, 2 and one stimulants respectively ; none of them was entirely negative.

Blood leukocyte counts: The initial mean blood leukocyte count was 11195 ± 3055 in 12 CM patients and 7975 ± 2845/c.mm in 38 NCM patients. The mean blood lymphocyte number was 3440 ± 1390/c.mm in CM patients and 2540 ± 1015/c.mm in NCM patients ($p > 0.05$). A lymphocyte count equal to or lower than 1200/c.mm was observed in 2 of 11CM patients and 4 of 32 NCM patients. Lymphocytes were over 4000/c.mm in 5 of 11 CM and 3 of 32 NCM patients. No major alteration of the number of circulating T and B cells

was observed : AET-E reactive T cells were present at an average concentration of 2050 ± 1300/c.mm in 8 CM and 1680 ± 80/c.mm in 28 NCM patients. The mean number of membrane immunoglobulin bearing lymphocytes was 600 ± 220/c.mm in 6 CM and 460 ± 250/c.mm in 12 NCM patients.

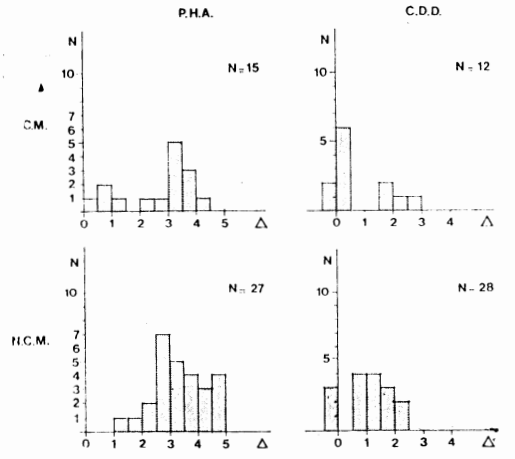

Fig. 1—Distribution of individual lymphocyte proliferative responses to phytohaemagglutinin (PHA) and candidin (CDD) in CM and NCM patients.

Initial *in vitro* lymphocyte response to mitogens and antigens: Fig. 1 shows the distribution of *in vitro* proliferative responses to PHA, and CDD. Three of 15 CM patients exhibited diminished proliferative response more than 2 SD from the mean response of all patients (mean = 3.081 ± 1.045) whereas none of 27 NC patients did. In all patients with diminished responses to PHA, decreased responses to ConA was simultaneously observed. There a no significant difference in the proliferative responses to PWM between CM and NCM patients. *In vitro* proliferative responses to CDD were recorded in 10 of 12 CM and 25 of 28 NCM patients.

Evolution in the course of malaria: After therapy, parasitaemia became lower than 1 per cent within two or three days in all cases. No major alteration of the proportion of AET-E reactive cells was observed during the follow-up period. The reappearance of delayed skin reactions to soluble antigens was detected four to eight days later in two CM and four NCM patients (to CDD only in two, to SKSD only in one and to both antigens in three cases) 4 to 8 days post admission in most instances. No suppression of proliferative responses was observed even in patients with negative cutaneous responses to PHA. The *in vivo* and *in vitro* responses to candidin were found to be correlated, whereas such responses to PHA and SKSD were not.

DISCUSSION

In the present study, the existence and nature of the cellular immunodeficiency associated with falciparum malaria was investigated. No correlation was found between initial parasitaemia and the presence or absence of neurological involvement. Decreased or abolished delayed cutaneous responses to PHA and soluble antigens was the major abnormality observed. This was consistently found in CM cases and was associated with high parasitaemia in NCM cases. Negative cutaneous responses to PHA appeared to reflect the immunodeficiency state better than other soluble antigens. Skin reactions to CDD were positive in the majority of healthy controls, thus confirming the previous reports from Thailand (Edelman *et al.*, 1973).

There was no evidence of consistent modifidation of the level of circulating T or B lymphocytes. *In vitro* proliferative responses of lymphocytes to lectins were found to be within normal ranges in most patients. A significant decrease of responses to conA has been found in several cases but a diminished response to PHA was observed only in three cases.

The present data confirmed the previous reports on T cell reactivity in human malaria.

In African patients with low parasitaemia, normal delayed skin responses were observed (unpublished). A significant but transient decrease in the number of circulating T cells in falciparum malaria cases was reported in a study of 33 West African patients (Wyler, 1976) and 36 Nigerian children (Greenwood *et al.*, 1977) with parasitaemia ranging from 0.02-6 per cent and 1-20 per cent respectively. In another study in Thai patients with parasitaemia lower than 1.1 per cent it was concluded that a significant but limited effect on the number of circulating T cells was exerted by the parasites (MacDermott *et al.*, 1980). We did not confirm this observation in cases with a 50 times higher parasitaemia. However, this discrepancy may be related to the use of untreated sheep erythrocytes by these authors, whereas AET-E rosettes were studied in the present report. Although a small decrease of proliferative responses to lectins was found in some studies (Osunkoya *et al.*, 1972), normal figures were reported in other studies (Greenwood *et al.*, 1972 ; MacDermott *et al.*, 1980 ; Ballet *et al.*, 1982). From our results and previous reports from others, it can be concluded that acute *P. falciparum* infection is able to suppress delayed cutaneous reaction, particularly to soluble antigens, and the *in vitro* responses to soluble antigens, but their suppressive effects on the number of T cells and their proliferative responses to lectins appear to be uncommon or limited.

SUMMARY

In Thai patients with acute *P. falciparum* malaria including cerebral cases, cell mediated immune functions were studied *in vivo* and *in vitro*. Initial cutaneous delayed reactions to phytohaemagglutinin and soluble protein antigens were negative in most cerebral malaria patients. No major alteration of the number of circulating T and B cells was observed. In lymphocytes cultures, proliferatives responses to lectins or protein antigens were generally found within normal ranges. This study shows a direct role of *P. falciparum* on the impairment of cell mediated immunity.

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