

EXAMINATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS AND  
SERA FROM THAI ADULTS NATURALLY INFECTED WITH  
MALARIA IN ASSAYS OF BLASTOGENIC RESPONSIVENESS  
TO MITOGENIC LECTINS AND ALLOGENEIC  
CELL SURFACE ANTIGENS

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OBJECTIVE : To examine the functional capabilities of peripheral blood cells from patients with naturally acquired malaria and to also examine the effect of their sera on cellular immune function using the mitogen induced lymphocyte transformation assay and mixed leukocyte culture system.

BACKGROUND : Delineation of the host immune response to infection with malaria should include examination of infected humans. Studies in this area have only recently begun to be carried out utilizing peripheral blood mononuclear cells (MNC) from patients. We have previously observed that Thai adults naturally infected with either *P. falciparum* or *P. vivax* have a decrease in the percentage and concentration of T lymphocytes, an increase in the percentages, but no change in the concentrations of B and "Null" lymphocytes, and no change in either the percentage or concentration of Fc receptor bearing lymphocytes (6). Thus, peripheral blood MNC from patients who have malaria exhibit a true loss of T cells without any real change in B cells, Fc receptor bearing cells, or Null cells. In addition, we have recently demonstrated that sera of Thai adults naturally infected with both *P. falciparum* and *P. vivax* contain cold reactive lymphocytotoxic antibodies with marked reactivity at 15°C (7). A number of individuals also had lymphocytotoxic antibodies which were effective at 37°C. Although the subpopulations against which these antibodies are directed have not yet been elucidated, the ability of serum components to interact with peripheral blood MNC in functional assays clearly needs to be ascertained. Therefore, in order to examine the functional capabilities of peripheral blood cells from

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patients with malaria and to also examine the effects of their sera on cellular immune function, we have begun to examine the peripheral blood MNC and sera of Thai adults naturally infected with *P. falciparum* and *P. vivax* in a number of *in vitro* cellular immune assays. The present work describes our results using mitogen induced lymphocyte transformation and mixed leukocyte culture systems.

## MATERIALS AND METHODS :

Patients : Peripheral blood was obtained from normal volunteers, or naturally infected Thai adults (ages 16 to 50) with *P. falciparum* or *P. vivax*. The patients were mildly ill, recently infected, outpatients from the region surrounding Phrabuddabat, which is endemic for malaria. Individual serum samples were also obtained from infected Thai adults or from uninfected individuals (controls) living in the same regions as the patients. No infected or control individuals donating cells or serum had a history of receiving blood transfusions, and none were on medication. The degree of parasitemia in all infected individuals was assessed by examination of Giemsa stained peripheral blood smears. The percent parasitemia values for *vivax* patients ranged between "positive" and 1.0% while those for *falciparum* patients ranged between "positive" and 1.1%. Patients were then treated by the staff of the National Malaria Project.

Mononuclear Cell Isolation : Peripheral blood mononuclear cells (MNC) were isolated from heparinized blood by diluting the blood 1:2 in Hanks' balanced salt solution (HBSS, GIBCO, New York) followed by ficoll-Hypaque centrifugation (1).

Lectin Induced Mitogenesis and Recognition of Allogeneic Cell Surface Antigens : The method used for analysis of lectin induced blast transformation has been reported previously by us (4). Methods used for the allogeneic mixed leukocyte reaction (MLR) have also been described previously by us (3).

Statistics : The student's t test was used to determine statistical significance, and a p value of less than 0.05 was considered significant.

## RESULTS :

Examination of Patient Peripheral Blood Mononuclear Cells : We first examined the ability of peripheral blood mononuclear cells (MNC) from patients infected with *P. falciparum* or *P. vivax* to respond to selected mitogens in culture. The responsiveness of patient peripheral blood MNC to Con A, PHA and PWM is summarized in Table 1. The responsiveness of the patient cells was equal to that of normal controls. Thus, with regard to stimulation by mitogenic lectins, peripheral blood MNC from mildly ill patients naturally infected with malaria did not show a decreased responsiveness.

We next turned our attention to the allogeneic mixed leukocyte reaction (MLR) in order to examine the capacity of infected patient's MNC to respond to or stimulate allogeneic cells from individual normal volunteers. As can be seen in Table 2, when cells from patients with *P. falciparum* were used as responders in MLR, a normal response to cell surface antigens on allogeneic normal cells was observed. In contrast, MNC from *P. vivax* patients had a

statistically significant decreased responsiveness ( $p < .05$ ) in allogeneic MLR. When cells from patients with malaria were used as stimulators in the MLR, individual normal responding cells exhibited significantly ( $p < .05$ ) decreased blast transformation. Thus, although MNC from malaria patients functioned normally in response to mitogens, *P. vivax* MNC were abnormal as responders in MLR and both *P. vivax* and *P. falciparum* MNC were suboptimal in their capabilities to function as stimulators in the one-way allogeneic MLR.

Modulation of Normal Mononuclear Cell Function by Patient Sera : We next examined the effects of sera from patients with malaria on mitogenic responsiveness and on the MLR using normal human MNC from single individuals as the indicator cells in both systems. As can be seen in Table 3, in experiments using 20% pooled sera from patients with *P. falciparum* or *P. vivax*, the mitogenic responsiveness of normal peripheral blood mononuclear cells was markedly reduced to both PHA and Con A. There was no statistically significant decrease in the mitogenic responsiveness to PWM. We also investigated the effect of individual sera from patients with malaria on responsiveness to the mitogens and the results were similar to that seen with the pooled sera.

Finally, we also studied the effect of 20% pooled sera from *P. falciparum* patients on normal allogeneic cells in MLR. These results are displayed in Table 4. The patient sera decreased the stimulation index from 9.9 to 5.1. Thus, sera from patients with *P. falciparum* appeared to have an inhibitory effect on the normal blastogenic response to allogeneic cell surface antigens *in vitro*.

Table 1. Responsiveness of Peripheral Blood Mononuclear Cells from Normals and Malaria Patients to Mitogens

	Con A		PHA		PWM	
	$\Delta$ cpm	S.I.	$\Delta$ cpm	S.I.	$\Delta$ cpm	S.I.
<i>P. vivax</i> (9)	27,068 $\pm 2,511^a$	41 $\pm$ 9	65,620 $\pm 3,758$	91 $\pm$ 15	31,853 $\pm 3,228$	49 $\pm$ 12
Controls (9)	28,993 $\pm 2,603$	39 $\pm$ 4	61,471 $\pm 4,457$	89 $\pm$ 16	29,349 $\pm 4,954$	33 $\pm$ 5
<i>P. falciparum</i> (6)	23,562 $\pm 2,001$	34 $\pm$ 5	53,957 $\pm 3,279$	74 $\pm$ 6	26,092 $\pm 2,333$	37 $\pm$ 4
Controls (6)	28,711 $\pm 1,962$	49 $\pm$ 7	57,537 $\pm 3,127$	100 $\pm$ 17	27,508 $\pm 2,528$	49 $\pm$ 10

<sup>a</sup> Mean stimulation index  $\pm$  standard error of mean for number of experiments in parenthesis.

Table 2. Responsiveness of Peripheral Blood Mononuclear Cells from Normal Individuals and Malaria Patients in One Way Mixed Lymphocyte Cultures<sup>a</sup>

	<u>Normal Responders</u>	<u>Patient Responders</u>
Patients infected with <i>P. vivax</i> (22) :		
Normal stimulators	8.5 ± 1.6 <sup>b</sup>	5.2 ± 0.9
Patient stimulators	5.9 ± 0.9	N.D. <sup>c</sup>
Patients infected with <i>P. falciparum</i> (22) :		
Normal stimulators	11.3 ± 2.2 <sup>d</sup>	12.2 ± 3.2 <sup>e</sup>
Patient stimulators	5.1 ± 1.0	N.D.

<sup>a</sup> Individual responder and individual stimulator cells were incubated 120 hrs at 37°C, pulsed an additional 24 hrs with <sup>3</sup>H-thymidine, and stimulation index determined. Results expressed as mean stimulation ± S.E.M. for number of experiments (each a separate patient) performed (in parentheses).

<sup>b</sup> Value obtained with normal responders and stimulators was significantly different from values obtained with normal responders and patient stimulators (p < 0.5) or patient responders and normal stimulators (p < .05).

<sup>c</sup> Not done.

<sup>d</sup> Value obtained with normal responders and stimulators was significantly different from value obtained with normal responders and patient stimulators (p < .02).

<sup>e</sup> Value obtained when patient's cells were used as responders was significantly different from value obtained when patient's cells were used as stimulators (p < .05).

Table 3. Effect of Pooled Sera from Patients (10 *P. vivax* and 10 *P. falciparum* Patients) on Responsiveness of Normal Peripheral Blood Mononuclear Cells to Mitogens

Pooled Sera	Phytohemagglutinin	Concanavalin A	Pokeweed Mitogen
<i>P. vivax</i> (15)	81 ± 9 <sup>a</sup>	53 ± 10	74 ± 13
Normal Controls (15)	160 ± 15	127 ± 20	105 ± 23
Statistical Significance	p < .001 <sup>b</sup>	p < .01 <sup>b</sup>	p < .3
<i>P. falciparum</i> (16)	81 ± 9	69 ± 9	86 ± 12
Normal Controls (16)	157 ± 9	112 ± 11	81 ± 14
Statistical Significance	p < .001 <sup>b</sup>	p < .01 <sup>b</sup>	p < .9

<sup>a</sup> Mean stimulation index ± standard error of the mean for the number of individual experiments in parenthesis.

<sup>b</sup> Statistically significant difference.

TABLE 4. Effect of Pooled Sera from 10 *P. falciparum* Patients on Responsiveness of Normal Peripheral Blood Mononuclear Cells in the Allogeneic Mixed Leukocyte Reaction (MLR)

Pooled Sera	Stimulation Index of MLR
Normal (44)	9.9 ± 1.4 <sup>a</sup>
<i>P. falciparum</i> (12)	5.1 ± .6
Statistical Significance	p .01 <sup>b</sup>

<sup>a</sup> Mean stimulation index ± standard error of the mean for the number of experiments in parenthesis.

<sup>b</sup> Statistically significant difference.

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