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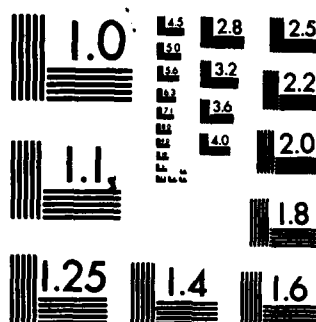
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CELLULAR IMMUNE MECHANISMS IN MALARIA

ANNUAL REPORT

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by

Richard P. MacDermott, Jr., M.D.

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Fort Detrick, Frederick Maryland, 21701

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Abstract

The cell-mediated immune defense mechanisms responsible for host resistance to malaria are poorly understood. We therefore examined peripheral blood mononuclear cells and serum from Thai adults naturally infected with malaria in order to determine: 1) the percentages of T, B, and Fc receptor bearing cells present during active infection, 2) the functional competence of peripheral blood lymphocytes from infected individuals as judged by responsiveness to mitogens and cell surface antigens, 3) the effect of serum from infected patients on normal lymphocyte function, and 4) whether or not there were anti-lymphocytotoxic antibodies present in the sera of malaria patients. In comparison to healthy controls, both the percentage and concentration of peripheral T cells were decreased in individuals infected with P. falciparum and P. vivax. The percentage of peripheral B cells was increased but their concentration was unchanged. Both the percentage and concentration of lymphocytes bearing Fc receptors were unchanged in infected individuals. Peripheral blood mononuclear cells exhibited normal responsiveness to the mitogens PHA, Con A, PWM, and to allogeneic cell surface antigens despite the decrease in T cell number. Sera from patients infected with malaria inhibited PHA and Con A but not PWM induced mitogenesis by normal cells. The sera also suppressed mixed lymphocyte reactivity by normal lymphocytes. Finally, anti-lymphocytotoxic activity was found in the sera of 95% of both P. falciparum and P. vivax infected Thai natives. Thus, in summary: 1) in adult Thai patients naturally infected with

malaria, there is a real loss of circulating T lymphocytes with no real change in B or Fc receptor bearing lymphocytes and with no loss of lymphocyte function as judged by mitogenic and antigenic stimulation; 2) sera from infected patients is abnormal in that it suppresses functional capacities of normal lymphocytes; and 3) sera from patients with malaria contain antibodies capable of destroying normal lymphocytes. Future studies will be necessary to determine whether the anti-lymphocytotoxic activity is related to either the functional suppression or loss of circulating T cells.

Introduction

A better understanding of cell mediated immune mechanisms in malaria may allow more rational approaches to ways of improving immunological defense mechanisms. Therefore, it is hypothesized that the functional characterization of subpopulations of lymphocytes in patients clinically infected with malaria and characterization of the immune modulatory capabilities of serum from these patients will provide insight into the control and effector functions of the cellular immune system's role in host resistance to malaria.

It has been well demonstrated in animals, that circulating antibodies can be passively transferred and will provide protection against malaria (1-3). In addition, the transfer of resistance with cells has been demonstrated for P. berghei in rats (4,5) with a subsequent study that the most likely mechanism by which cell transfer worked was related to antibody production (6). Thus, based on animal studies, lymphocytes are most likely involved in protection against malaria by virtue of their ability to synthesize and secrete antibody and to regulate antibody production. However, the immune response to an active malaria infection in humans is not completely understood.

The changes in lymphocyte populations have been studied in African children infected with P. falciparum (7,8) but it is unclear as to whether these findings can be generalized for other malaria parasites to infected adults living in other areas or what the functional significance of lymphocyte alterations might be. For this reason, we chose to determine the percentages of T, B, and Fc receptor bearing cells

and the blastogenic response of unseparated cells to mitogens and cell surface antigens in Thai adults naturally infected with P. falciparum and P. vivax.

In addition, we chose to examine the effect of serum from malaria patients on normal lymphocytes with regards to modulation of mitogen and cell surface antigen induced blastogenesis. These studies were begun on the premise that extrinsic factors as well as intrinsic alterations might be important in regulating the immune response to malaria.

One of the major control mechanisms in cellular immune function may be anti-lymphocytotoxins. Antibodies directed against white blood cells were first noted to occur after blood transfusions (9). Subsequent studies revealed that the serum from 17% of multiparous women contained antibodies directed against leukocytes (10). These observations led to the development of the current tissue typing reagents which employ serologically defined (SD) anti-lymphocytotoxic antibodies (11). Further investigations disclosed that both transplantation of allogeneic skin grafts (12) and intradermal injection of allogeneic leukocytes (13) would lead to antibodies directed against white blood cells. All of these above studies, therefore demonstrated the ability of an individual to produce antibodies to histoincompatible cell surface antigens.

More recently, attention has focused on the spontaneous induction of autologous and allogeneic anti-lymphocytotoxic antibodies. The initial studies in this area demonstrated the presence of anti-lymphocytotoxins in patients with infectious mononucleosis, rubella, and measles (14).

Of particular interest was that these antibodies were optimally detected at 15° C. Shortly thereafter, similar antibodies were detected in systemic lupus erythematosus (15). Subsequently, a variety of other disease states have been shown to be associated with an increased incidence of anti-lymphocytotoxic antibodies, including inflammatory bowel disease (16) and multiple sclerosis (17). We therefore decided to investigate sera of adult Thai patients naturally infected with either P. falciparum or P. vivax for warm and cold reactive anti-lymphocytotoxic antibodies.

Methods

A. Lymphocyte subpopulation enumeration and functional characterization:

Isolation of mononuclear leukocytes:

Peripheral blood mononuclear leukocytes (MNL) were obtained according to the method of Boyum (18). Heparinized blood was drawn from each malaria patient immediately before treatment or 14 days after the initiation of treatment. The blood was diluted 1:2 in Hanks Balanced Salt Solution (HBSS, Grand Island Biological Co. (GIBCO) New York) and layered on Ficoll-Hypaque (Pharmacia Fine Chemicals, New Jersey). Following centrifugation, the MNL were removed by Pasteur pipette from the interface. The MNL were then adjusted by hemocytometer count to a concentration of 2×10^6 cells in HBSS.

Preparation of sheep red blood cells for rosette forming assays:

Sheep red blood cells (SRBC) in Alsever's solution were filtered with sterile gauze and washed with thriethanolamine-buffered salt solution (TBS). The SRBC were resuspended at a concentration of $7-8 \times 10^9$ cells/ml in TBS containing 0.1% gelatin (Baltimore Biological Laboratory, Baltimore, Maryland). Unmodified SRBC (E rosettes) were used to determine the percentage of T cells. In order to detect cells with Fc receptors, SRBC were coated with subagglutinating amounts of 7S (IgG) anti-SRBC (Cordis Laboratories, Miami, Florida) to form EA rosettes. In order to detect cells bearing complement receptors, SRBC were coated with subagglutinating amounts of 19S (IgM) anti-SRBC (Cordis) and then incubated with fresh mouse serum.

Procedures for detecting rosette forming lymphocytes:

The methods of Mendes et al were employed with modifications as previously described (19). The percentage of cells forming E rosettes were determined after 5 minutes, 1 hour, and 18 hours (overnight) at 4° C. The percentage of cells forming EA and EAC rosettes was determined after 30 minutes at 37° C. In calculating the percentages of lymphocytes forming rosettes with three or more red cells, both sides of a hemocytometer chamber were counted and the values of rosetting and nonrosetting lymphocytes were averaged.

Direct fluoresceinated antibody technique:

Surface immunoglobulin bearing B lymphocytes were also identified by staining with fluorescein labelled anti-human-immunoglobulin (GIBCO). The methodology of Chess et al (19) was employed with modification. Briefly, $3-4 \times 10^6$ MNL were incubated for 1 hour at 37° C to remove non-specifically adsorbed immunoglobulin (20), washed three times, centrifuged, and fluoresceinated anti-human immunoglobulin added. After mixing and incubation, the cells were again centrifuged and washed in HBSS containing 10% heat inactivated fetal calf serum (GIBCO). After final centrifugation, the cells were resuspended in cold glycerol phosphate buffered saline and the percentage of fluorescing cells determined using a Leitz ortholux microscope equipped with BG 12 and K 530 filters with a 220 watt high pressure mercury lamp. As with the rosette assays, counts were done blind with the sample origin unknown, readings were done in duplicate, and the results were averaged.

Mitogen and cell-surface antigen induced blastogenesis:

The proliferative response of peripheral blood lymphocytes to mitogens was investigated by standard techniques as we have previously described (21-23). In brief, 1×10^5 cells for mitogen studies were placed in triplicate in microtiter plates with either media, phytohemagglutinin, concanavalin A or pokeweed mitogen. Cultures were pulsed after 4 days with 0.05 ml of media containing 0.2 μ Ci methyl- 3 H-thymidine. After 4 hours in culture with the thymidine, the cells were harvested using a MASH II extractor. One-way mixed lymphocyte cultures (MLC) were performed as previously described (21,23). In brief, 2×10^5 responding cells and 2×10^5 mitomycin-C treated stimulating cells in 0.2 ml of final culture medium were placed in triplicate in microtiter plates. After 6 days, the cultures were pulsed with 0.2 μ Ci of 3 H-thymidine for 16 hours and harvested with a MASH II apparatus.

B. Serum studies:

Patients studied: Individual serum samples were obtained from 58 Thai adults naturally infected with P. falciparum and 50 with P. vivax. Sera was also obtained from 37 normal healthy individuals living in the same region as the infected patients. None of the infected or control individuals had a history of receiving blood transfusions and none were on medications. To further reduce the possibility that any anti-lymphocytotoxic antibodies detected might be due to causes other than malaria infection, only male patients or females with no more than a single pregnancy were accepted into the study. The degree of parasitemia was assessed by a Giemsa stained peripheral blood smear. After the blood was clotted at room temperature, the serum was removed and stored at -20° C.

The patients participating in this study were then treated by the staff of the National Malaria Eradication Center, Sri Racha, Thailand.

Anti-lymphocytotoxic antibody assay: Cytotoxic assays were run in duplicate in flat bottomed tissue culture trays (Costar Cluster 96, Cambridge, Massachusetts). The methodology was modified from previously described techniques (15). In brief, peripheral blood mononuclear cells (PBC) were isolated according to the method of Boyum (18). After three washes in Hanks Balanced Salt Solution (HBSS), the cells were adjusted to a final concentration of $3 \times 10^6/\text{ml}$ in HBSS containing 10% heat inactivated fetal calf serum and 0.1 ml added to each well of the culture tray. Subsequently, at either 15°C or 37°C , 0.1 ml of each sera (in duplicate) was added to appropriate wells for 30 minutes followed by addition of 0.1 ml of fresh rabbit serum as a source of complement. After an additional 4 hours at either 15°C or 37°C , the percentage of dead cells was determined by eosin dye inclusion. Twenty-five different normal healthy lab personnel served as donors of the PBC's used as indicator target cells for the anti-lymphocytotoxic antibodies. Both control sera and patient sera were run simultaneously. Sera from a patient with systemic lupus erythematosus served as a positive control and killed an average of 34% of the target PBC's. Reproducibility of the assay (checked by repeating the same sera directed against the same targets at different times) demonstrated essentially identical results for the 10 sera tested.

Serum inhibitory studies: The effect of serum from infected patients on mitogen and cell surface antigen induced blastogenesis by normal lymphocytes was assessed by placing individual (20%) or pooled sera (20%) from patients

in the mitogenesis assays described above using normal PBL's instead of patient PBL.

Treatment of patients: The treatment of malaria patients participating in these studies was administered by the staff of the National Malaria Eradication Project at Pradubbhabaht, Thailand. Patients infected with P. falciparum were given 1 gram of sulfadoxine (Fansidar) and 50 mg pyrimethamine in a single dose and 15 mg primaquine/day for 4 days. Patients with P. vivax infections were treated with 1500 mg chloroquine and 15 mg primaquine/day for 5 days.

Statistical analyses: The Student's t test was used and $p < .01$ was considered necessary to obtain "significance".

Results

A. Lymphocyte subpopulation enumerations and functional characterization:

Forty-nine patients infected with either P. falciparum or P. vivax were studied. The 24 P. falciparum patients ranged in age from 17 to 48 years (mean 25). Twenty-five patients with P. vivax were studied, who ranged in age from 16 to 45 years (mean 24). The patients were inhabitants of the region surrounding Prabuddabaht which is endemic for malaria. All of these patients indicated that they had had one or more previous episodes of malaria but no records were available to validate their history. A group of 21 healthy Thais with no previous history of malaria served as controls. These individuals ranged in age from 19 to 45 years (mean 32) and were of similar ethnic background and geographic area as the study group.

Circulating lymphocyte subpopulations were studied by E, EA and EAC rosette formation and fluorescein labelled anti-human-immunoglobulin (FITC) staining and the results expressed either as a percentage (Table 1) or as an absolute concentration (Table 2). To determine the percentage and concentration of T lymphocytes, the E rosette technique was employed with incubation times of 5 minutes, 1 hour and 18 hours. The 5 minute values indicated that there was a marked suppression in the percentage (Table 1) and concentration (Table 2) of T cells in both P. falciparum and P. vivax patients. There was likewise a reproducible pattern of a decreased percent and concentration of E rosette forming cells (T lymphocytes) in patients with malaria, at assay times of 1 hour and 18 hours (Tables 1 and 2). The decrease in percent and concentration of E rosette forming lymphocytes

(T cells) was statistically significant in 10 of the 12 comparisons (Tables 1 and 2).

The EA rosette technique was utilized in evaluating the percentage of circulating Fc bearing cells. Patient cells showed virtually identical mean values in comparison with normal controls and no significant change in terms of either percentage (Table 1) or concentration (Table 2) of Fc bearing cells was observed. Application was made of the EAC rosetting technique and the FITC staining technique in identifying circulating B lymphocytes. The mean EAC values were 25% for patients with falciparum malaria as compared with 24% for the vivax patients and 16% for the normal controls. The FITC cell values for patients were likewise elevated and showed excellent correlation with those for the EAC (B cell) rosettes with mean percentages of 24 for falciparum patients, 22 for vivax patients and 15 for the normal group. The elevated percentages of B cells in the patients compared to controls was statistically significant ($p < 0.01$) for both techniques. However, when the concentration of B cells was calculated, there was essentially no difference found in the number of B cells in patients with malaria in comparison to normal controls (Table 2).

Because of the decrease in circulating T lymphocytes noted during malaria infection, described above, we have begun to investigate abnormalities in lymphocyte function in Thai patients naturally infected with malaria. We started our studies by examining in vitro mitogen induced lymphocyte responsiveness as a general assessment of T cell function and allogeneic mixed lymphocyte culture as a specific T cell

functional characteristic. Our data has demonstrated that lymphocytes from adult Thai patients with P. vivax and P. falciparum exhibit normal responsiveness to phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM). Likewise, normal responsiveness in mixed lymphocyte culture was observed.

B. Serum studies:

Sera from 58 patients with P. falciparum, 50 patients with P. vivax, and 37 normal healthy controls were examined for the presence of anti-lymphocytotoxic antibodies in assays run at 15° C and 37° C. There was a wide range of anti-lymphocytotoxic activity exhibited by the individual sera, causing from 2% to 78% of the target mononuclear cells to be lysed (Figure 1). As seen in Table 3, the killing due to control sera averaged only 3.2% (15° C) and 3.3% (37° C). None of the control sera lysed more than 5% of the indicator cells (Figure 1). When sera from patients with either P. falciparum or P. vivax were examined, marked anti-lymphocytotoxic activity was observed (Figure 1, Table 3). If the mean plus two standard deviations of the control value is used as an upper limit of normal in studies done at 15° C, 98% of P. vivax patients and 95% of P. falciparum patients had antibodies in their sera which were cytotoxic for a greater percentage of indicator lymphocytes than would normally be expected (Figure 1). The average percent of indicator cells was significantly greater for serum from patients with vivax and falciparum malaria than controls ($p < .001$; Table 3). The anti-lymphocytotoxic antibodies were significantly more reactive using 15° C as the incubation temperature than using 37° C (Figure 1, Table 3, $p < .001$).

Furthermore, this temperature dependent aspect held for individual sera investigated, in that the sera which exhibited greater reactivity at 15° C also exhibited greater reactivity at 37° C. There was no relationship between the percent of indicator mononuclear cells killed and the patient's degree of parasitemia, age, or sex.

Because of the depressed numbers of T cells yet normal T cell functional capacity as judged by responsiveness to soluble mitogens and cell surface antigens we next examined the effect of patient's serum on the functional capabilities of normal lymphocytes. We have determined that patient sera, both pooled and individual samples, depresses normal lymphocyte responsiveness to allogeneic cell surface antigens (mixed lymphocyte culture), PHA, and Con A but does not interfere with PWM induced blastogenesis.

Discussion

We have 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 percentage but no change in the concentration of B lymphocytes and no change in either the percentage or concentration of Fc receptor bearing cells. However, T cell function, as judged by lymphocyte responsiveness to mitogenic lectins and to cell surface antigens appears to be intact despite the decrease in T cell number and concentration.

These findings are in agreement and disagreement with various aspects of previously reported results by Wyler (7), who studied 30 children and 3 adults in West Africa with P. falciparum, and Ade-Serrano and Osunkoya (8), who also studied children with P. falciparum. Both previous studies (7,8) and the present study found that during acute infection with malaria, the percentage of cells forming E rosettes (T cells) was decreased. Both the study of Wyler (7) and the present study also found a decrease in the concentration of T cells, thus signifying a true loss of T cells. Both previous studies (7,8) found similar results to the present study in observing an increase in the percentage of B cells using EAC rosettes. More importantly Wyler (7) found, as we did, that there was no increase in the concentration of B cells.

While the T cell depression observed in infected patients could represent a transient relocation of lymphocyte pools, it would be reasonable to hypothesize that the loss of T cells could be due to destruction of a specific T cell subclass. Furthermore, the ability of

T cells or Fc receptor bearing cells to participate in cellular cytotoxic reactions might be enhanced or depressed during active infection due to the changes in cell populations. Whatever the underlying mechanism(s), the depression in numbers of T lymphocytes may not be limited to circulating cell populations but may involve T cell depletion of the lymphoid organs as well. Indeed, Krettli and Nussenzweig reported depletions in mouse lymphocyte populations in thymus glands and lymph nodes during infection with P. berghei (24).

Following the classification of the changes occurring in relative proportions of lymphocyte subpopulations as observed above we have begun to study the functional competence of the patient's lymphocytes. In particular, the loss of T cells in the patients raises the question of which T cell functions might be lost. The observations above that peripheral blood lymphocytes from patients with malaria will undergo mitogenesis induced by lectins and cell surface antigens (mixed lymphocyte culture) demonstrates that T cells from infected patients are able to be non-specifically stimulated and specifically sensitized in vitro. The possibility was therefore raised that the decrease in T cell number was related to serum factors. Our studies next demonstrated that pooled and individual serum samples from P. falciparum and P. vivax patients have an inhibitory effect on allogeneic cell surface antigen, PHA, and Con A induced mitogenesis but not on PWM induced mitogenesis. Since cellular competence with regards to mitogenesis does not necessarily reflect cellular effector competence future studies will be needed in order to examine the ability of patient T cells to kill in cell mediated

lymphocytotoxicity and of patient Fc receptor bearing cells to function in antibody dependent cellular cytotoxicity. However, these studies raise the possibility that abnormalities of cellular immune function may be due to potentially reversible extrinsic serum factors rather than due to permanent intrinsic changes in lymphocyte functional capabilities. One of the serum factors that could be involved in immunoregulation would be anti-lymphocytotoxic antibodies. Indeed, we observed that sera from Thai adults naturally infected with either P. falciparum or P. vivax contain cold reactive (15° C) anti-lymphocytotoxic antibodies.

There is evidence from previous studies that active malaria interferes with a normal immune response. First of all, in mice infected with malaria, there is suppressed antibody production, reduced severity of autoimmune disease, and increased susceptibility to tumor viruses (25). Secondly, there is the immunopathologic effect of antigen-antibody complexes causing nephrotic disease in young children with P. malariae (26,27). Thirdly, anemia due to increased phagocytosis of uninfected erythrocytes has been noted in animals, yet the mechanism of this increased destruction is unknown. In this regard, although antibodies to red blood cells have been found with increased frequency in patients with malaria, it is possible that they may be secondary to the fragmented red cells rather than causative of an autoimmune hemolytic process (25,27). Finally, increased levels of auto-antibodies directed against heart, thyroid, and gastric parietal cells associated with elevated IgM levels and high titers of malarial antibodies have been noted in immigrant and indigenous peoples in Uganda (28). Thus, infection of a host with malaria has been shown to result in a variety of adverse effects on and of the

immune system. Our studies have demonstrated an additional immunologic abnormality: antibodies directed against peripheral blood mononuclear cells.

Initial studies of anti-lymphocytotoxic antibodies demonstrated their presence in a variety of clinical syndromes, with viral and presumed autoimmune diseases predominating. A parasitic infestation, malaria, can now be included in the list of diseases which induce anti-lymphocytotoxic antibody formation. There are several possible ways in which infection with malaria might induce the production of anti-lymphocytotoxic antibodies. Firstly, the antibodies might actually be directed against malaria antigens and just be cross reactive against lymphocytes. Secondly, malaria antigens could have become bound to the lymphocytes, causing the antibodies to be formed against altered self antigens. Thirdly, the process could be due to the presence of an immunologic stimulus which nonspecifically induced anti-lymphocytotoxin production. Fourthly, the antibodies could be an important part of the protective host response to malaria by causing a deletion of suppressor cells in order to enable heightened cytotoxic or antibody effector functions. Future in vivo and in vitro studies will be needed in order to answer which of these possible explanations is correct.

The characteristics of the anti-lymphocytotoxic antibodies in sera from malaria patients have not been investigated as yet. Previous studies indicate that cold reactive lymphocytotoxic antibodies are predominately IgM and directed against T cells (29,30) in disease states. In this regard, it should be noted that cold reactive IgM anti-lymphocytotoxic antibodies directed against B cells occur in approximately 20% of normal

individuals (31). Thus, the anti-lymphocytotoxic antibodies in malaria patients are most likely directed against more than B cells because greater than 12% of target cells are killed by the majority of sera studied. Nevertheless, future studies will be needed to address the questions of the type of anti-lymphocytotoxic antibodies found in malaria patients and the cell types which they are directed against.

The question remains as to the in vivo relationship of these anti-lymphocytotoxic antibodies and the decreased T cell numbers found in the peripheral blood of actively infected patients. Although the antibodies are cytotoxic in vitro only at 15° C, it is possible that they function in vivo similarly to antibodies in cold agglutinin disease. That is, in cold agglutinin disease IgM antibodies fix to a red blood cell (RBC) in the cooler peripheral parts of the body, fix complement, and then elute off the RBC in the warmer parts of the body leaving a complement coated RBC to be phagocytosed at a later time (32). Furthermore, antibodies from different individuals with cold agglutinin disease vary as to the optimal temperature at which they act and thus the severity of the disease process appears to depend upon the characteristics of the particular individual antibody (32). Therefore, there could possibly be a variable effect of the anti-lymphocytotoxic antibodies in patients with malaria depending on the temperature at which each person's antibody is optimally functional. It is important, of course, to consider the other alternatives as to the relationship between the anti-lymphocytotoxic antibodies and reduced numbers of T cells: 1) The reduced T cells could actually have led to anti-lymphocytotoxic antibody production through loss

of a suppressor cell regulatory effect, or 2) there may be no relationship between the two and the T cell loss is actually due to other factors. Additional studies will be necessary in this area as well in order to determine the in vivo relevance of anti-lymphocytotoxic antibodies in patients with malaria.

An understanding of the role of antibodies directed against lymphocytes in patients with malaria will hopefully be achieved by future studies on the nature of the antibody, the type of cells against which it is directed, the mechanisms leading to its production, and its in vitro effects on immunologically functional cells.

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Table 1
Changes in the Relative Percentages of Peripheral Blood Lymphocyte
Subpopulations in Thai Adults with P. falciparum and P. vivax

	Percent of Peripheral Blood Lymphocytes Identified by					Surface Immunoglobulins
	5' E rosettes	1 hr. E rosettes	18 hr. E rosettes	EA rosettes	EAC rosettes	
Controls (N = 21)*	41 ± .9†	54 ± .9	63 ± .3	5 ± .3	15 ± .2	15 ± .5
<u>P. falciparum</u> (N = 24)	28 ± 1.0 (p < .001)‡	41 ± 1.0 (p < .001)	52 ± .5 (p < .001)	5 ± .5 (p < .9)	25 ± .5 (p < .001)	24 ± .6 (p < .001)
<u>P. vivax</u> (N = 25)	29 ± 1.0 (p < .001)	41 ± 1.0 (p < .001)	54 ± .7 (p < .001)	5 ± .3 (p < .9)	24 ± .5 (p < .001)	22 ± .5 (p < .001)

* N = Number of subjects studied.

† Mean ± S.E.M.

‡ p value for malaria infected group in comparison to control

Table 2

Changes in the Concentration of Peripheral Blood Lymphocyte Subpopulations
in Thai Adults with P. falciparum and P. vivax

	Number of Peripheral Blood Lymphocytes per mm ³ Identified by					Surface Immunoglobulin
	5' E rosettes	1 hr. E rosettes	18 hr. E rosettes	EA rosettes	EAC rosettes	
Controls (N = 21)*	1162 ± 475†	1486 ± 154	1750 ± 136	137 ± 17	447 ± 35	400 ± 36
<u>P. falciparum</u> (N = 24)	564 ± 56 (p < .02)‡	781 ± 69 (p < .001)	999 ± 85 (p < .001)	100 ± 13 (p < .1)	486 ± 38 (p < .5)	467 ± 34 (p < .2)
<u>P. vivax</u> (N = 25)	557 ± 46 (p < .01)	1086 ± 311 (p < .3)	1058 ± 96 (p < .001)	97 ± 10 (p < .05)	483 ± 37 (p < .5)	429 ± 33 (p < .5)

* N = Number of subjects studied

† Mean ± S.E.M.

‡ p value for malaria infected group in comparison to control

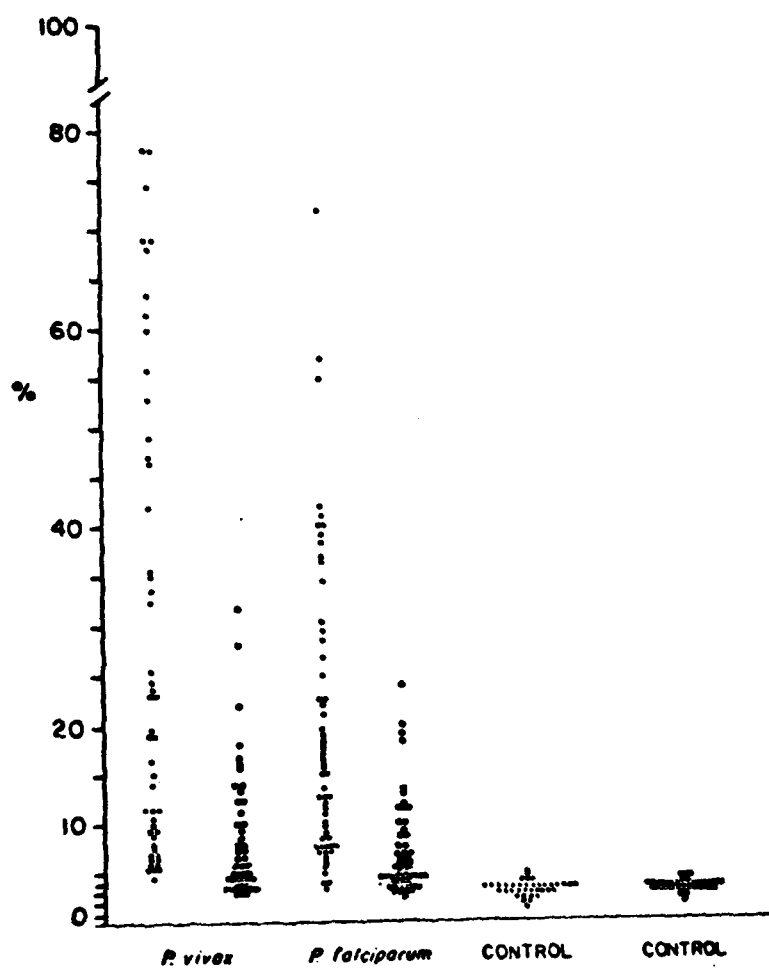
Table 3
Anti-Lymphocytotoxic Antibodies in Sera of Thai
Adults Infected with Malaria

<u>Sera</u>	<u>Percent Peripheral Blood Cells Killed</u>	
	<u>15° C</u>	<u>37° C</u>
Normal Control (37) ^A	3.2 ± 0.1 ^B	3.3 ± 0.1
<u>P. falciparum</u> (50)	19.9 ± 1.9	7.0 ± 0.7
<u>P. vivax</u> (58)	28.7 ± 3.3	8.5 ± 0.1

A: Number of individual sera examined in parenthesis

B: Mean ± 1 SEM

Figure 1



Antilymphocytotoxic antibodies in sera of Thai adults infected with malaria. Individual sera from patients with *P. falciparum*, *P. vivax*, and normal controls were examined at either 15° C or 37° C.