

DEFICIENCY OF CON A-INDUCED SUPPRESSOR CELL ACTIVITY IN  
PERIPHERAL BLOOD MONONUCLEAR CELLS FROM THAI ADULTS  
NATURALLY INFECTED WITH *Plasmodium falciparum* AND  
*Plasmodium vivax*

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OBJECTIVE : To study the suppressor cell activity in the peripheral blood mononuclear cells from patients with malaria.

BACKGROUND : In order to assess the cellular immunologic changes which occur in humans infected with malaria, we have begun to examine Thai adults naturally infected with either *Plasmodium falciparum* or *Plasmodium vivax* with regard to alterations of immune function. In our initial studies, we observed that both the percentage and concentration of peripheral blood T cells were decreased in malaria infected individuals as assessed by formation of rosettes with sheep red blood cells (Wells et al. 1979). The percentage of peripheral blood B cells was increased but their concentration was unchanged while both the percentage and concentration of peripheral blood Fc bearing lymphocytes were unchanged. Finally, although the percentage of Null cells in the peripheral blood was increased in infected individuals, calculation of the absolute number of Null cells revealed a decrease in this population (Wells et al. 1979). Therefore, we have found that in adult Thai patients naturally infected with malaria there is a loss of circulating T lymphocytes but no real change in B, Fc receptor bearing, or Null lymphocytes.

Because of the decrease in circulating T lymphocytes noted during malaria infection we next examined *in vitro* mitogen induced lymphocyte responsiveness in order to determine whether abnormalities in lymphocyte function might also occur. We found that peripheral blood mononuclear cells (MNC) from adult Thai patients with *P. vivax* and *P. falciparum* exhibited normal responsiveness to phytohemagglutinin (PHA), Concanavalin A (Con A) and pokeweed mitogen (PWM) (MacDermott et al. 1980). Furthermore, normal responsiveness in the mixed leukocyte reaction was observed with MNC from

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patients with *P. falciparum* (MacDermott et al. 1980). Therefore, despite the decrease in circulating T cells, responsiveness to mitogenic lectins and allogeneic cell surface antigens was intact.

Serum factors or antibodies might modulate the immune response to malaria infection, and we therefore have also examined sera from Thai patients with regard to the presence of lymphocytotoxic antibodies and suppressor factors capable of inhibiting mitogenesis or the mixed leukocyte reaction. Our studies have revealed that 98% of *P. vivax* patients and 95% of *P. falciparum* patients have antibodies in their sera which are cytotoxic for normal peripheral blood MNC (Wells et al. 1980). These lymphocytotoxic antibodies were found to be significantly more reactive at 15°C as opposed to 37°C (Wells et al. 1980). Although the precise reason for the development of lymphocytotoxic antibodies is unclear, it is possible that they could be an important part of feedback immunoregulatory pathways, as part of a modulation of the ongoing immune response to malaria infection. Indeed we also found that sera from patients with malaria do have an inhibitory effect on PHA and Con A induced mitogenesis (MacDermott et al. 1980).

Thus our studies to date have demonstrated that there are a number of major alterations in the immune system of patients naturally infected with malaria, including loss of circulating T cells, the presence of lymphocytotoxic antibodies, and the potential existence of serum suppressor factors. However, there is little information on the functional capabilities of lymphocyte subsets in malaria. Of course there is a particular need to understand the potential effector or regulatory mechanism alterations. We therefore have chosen in the present study to examine the ability of suppressor cells to be induced in peripheral blood MNC from malaria patients. We have found that there is decreased suppressor cell generation in both *P. falciparum* and *P. vivax* patients and thus hypothesize that one of the T cell subclasses which is lost during active malaria infection is the suppressor T cell precursor pool. These studies furthermore demonstrate that immunoregulatory abnormalities may arise secondary to a parasitic infection.

#### MATERIALS AND METHODS :

Patients : Thirty-two adult male patients (31 Thai, 1 Caucasian) with acute malaria (21 *P. falciparum*, 11 *P. vivax*) (age range, 17-35 years; mean 25.4) were studied. Among these, twenty-nine were regular patients attending the Malaria Eradication Center, Phrabuddabat, Thailand, while three were staff members of the Armed Forces Institute of Medical Sciences (AFRIMS), Bangkok, Thailand. All patients had low grade infection (< 0.5%) and only the AFRIMS staff (2 Thais, 1 Caucasian) were known to have recently taken medication; the remainder denied being on any medication.

Controls : Thirty-two healthy Thai adults were examined and at least one healthy control was tested in parallel with every patient.

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 (Boyum 1968).

Following three washings in HBSS, the isolated peripheral blood MNC were adjusted to a concentration of  $5 \times 10^6$  cells/ml in RPMI 1640 (GIBCO) assay medium containing 2 mM glutamine, 50 ug penicillin/ml, 50 ug streptomycin/ml, 25 mM N-2hydroxyethyl-piperazine-N2 ethanesulfonic acid (HEPES) buffer, and 20% heat inactivated human serum. Cell viability was determined by trypan blue dye exclusion; viability was greater than 95% in all specimens.

Identification of T-cells : T lymphocytes were identified by their property of forming rosettes with sheep red blood cells (SRBC). SRBC in Alsever's solution were filtered with gauze and washed with thriethanolamine-buffered solution (TBS). The SRBC were resuspended at a concentration of  $7-8 \times 10^9$  cells/ml in TBS containing 0.1% gelatin (Baltimore Laboratory, Baltimore, Maryland). The method of detecting rosette-forming cells has been reported previously (Wells et al. 1979). Briefly, the percentage of cells forming E rosettes was determined after 1 hr incubation at  $4^\circ\text{C}$ . In calculating the percentage of lymphocytes forming rosettes with three or more red cells, both sides of the haemocytometer chamber were counted and the values of rosetting and non-rosetting lymphocytes were averaged. Rosetting T cells were separated from non-rosetting MNC on a Ficoll-Hypaque gradient as described above. To obtain ERFC (T cells), the pellet was suspended in 0.83% ammonium chloride - 0.17 M Tris buffer, pH 7.2, centrifuged, washed twice in assay medium, and resuspended to the desired concentration in assay medium.

Con A activation of lymphocytes : The method of Shou, Schwartz & Good (1976) was used. Fresh unfractionated mononuclear cells or ERFC (T-cells) were adjusted to a concentration of  $3-5 \times 10^6$ /ml and incubated with or without Con A (6 ug/ml) in a humidified 5%  $\text{CO}_2$  incubator for 48 hours. At the end of incubation, the cells were washed three times, then treated with mitomycin C (25 ug/ $10^6$  cells) for 1 hour at  $37^\circ\text{C}$ , followed by four successive washes, and resuspended in RPMI at a concentration containing  $2 \times 10^6$  unfractionated MNC/ml or  $0.4 - 5 \times 10^6$  ERFC.

Cell cultures : Fresh mononuclear cells (responder cells) from autologous or allogeneic normal donors were prepared as described above but without Con A, and adjusted to a concentration of  $1 \times 10^6$  cells/ml. In one set of six experiments a single donor served as a source of responder cells. In the one-way mixed leukocyte reaction (MLR) experiments; Con A, mitomycin C (MC)-pretreated lymphocytes ( $2 \times 10^6$  cells/ml) from either malarious patients or controls were incubated with either autologous or normal allogeneic responder cells ( $1 \times 10^5$  cells/ml) for 5 days.

In the mitogen induced blast transformation experiments; 0.1 ml responder cells were mixed with an equal volume of either the patient or control Con A, MC-pretreated cell populations. Immediately after mixing the responder cells and the Con A, MC-pretreated lymphocytes, 25 ul of either phytohemagglutinin-P (PHA, 200 ug/ml Difco Laboratories), Concanavalin A (Con A 60 ug/ml, Calbiochem), or pokeweed mitogen (PWM, 1 mg/ml, GIBCO) were added to

appropriate wells and the plates incubated for 5 days. When allogeneic responder cells were used in the assays additional cultures were incubated without mitogen to determine the relative level of alloantigen stimulation in the individual experiments. To each well was added 0.4 uCi of (<sup>3</sup>H) thymidine (specific activity 40-60 Ci/mmol, New England Nuclear, Watham, MA) 18 hours prior to harvesting with a MASH II microtiter automatic cell harvester. Incorporation of (<sup>3</sup>H) thymidine into cells was measured by liquid scintillation counting. Results were expressed as the mean net counts of triplicate wells.

**Characterization of the systems :** In studies designed to establish the parameters of the experiment systems no difference was observed in the amount of suppression induced by pretreated cells washed with alphanethyl mannoside (α MM) and those washed with HBSS, subsequently cells were washed using only HBSS. Mitogenic concentrations of Con A (5 to 10 ug/ml) were found to be necessary to produce maximum suppression of blast transformation of responder cells. In addition, maximum suppression was observed when MNC were preincubated with Con A for 48 hours before being incubated in culture with responder cells for 5 additional days.

**Suppression :** The degree of suppression induced by Con A-pretreated cells preincubated without Con A on the response of fresh autologous or allogeneic cells to mitogenic stimulation was calculated according to the following formula :

Percent suppression =  $1 - \frac{\text{SI of cultures treated with Con A}}{\text{SI of cultures not treated with Con A}} \times 100$ . SI = CPM in presence of mitogen or allogeneic stimulator cells divided by the background CPM in the absence of mitogen or stimulator cells.

**Augmentation :** Augmentation occurs when cells preincubated with Con A enhance, rather than suppress, (<sup>3</sup>H) thymidine incorporation in responder cell cultures stimulated with allogeneic cells or mitogenic lectins. The mechanism of augmentation is unknown but may be due to an abnormal helper cell population masking suppressor cell activity in responder cell cultures.

## RESULTS :

In the studies performed, we examined the ability of Con A to induce suppressor cell activity in peripheral blood MNC from active, untreated Thai natives naturally infected with malaria in comparison to normal controls. The ability of suppressor cells to be generated by incubation for 48 hrs. with Con A was assessed using a variety of indicator systems including subsequent responsiveness of autologous or allogeneic MNC from malarious individuals or healthy control volunteers to the mitogenic lectins PHA, Con A and PWM, as well as the ability of the autologous or normal cells to respond to allogeneic cell surface antigens in the mixed leukocyte reaction. Table 1 depicts the subsequent responsiveness of peripheral blood MNC to PHA after incubation with autologous MNC that had been preincubated for 48 hours with Con A. As can be seen, normal controls were induced to exhibit marked suppressor cell activity after Con A induction, with a mean suppression of

62.6 ± 7.1% (mean ± S.E.M.). However, when peripheral blood MNC from patients with malaria were examined, a markedly reduced degree of suppression of autologous peripheral blood MNC was seen, with a mean suppression of only 18.4 ± 10.4%. The difference in suppressor cell generation capacity (suppressor cells able to be generated in normal controls but not in malaria patients) were statistically significant ( $p < .005$  compared to controls).

We also found similar results as shown in Table 2 using Con A as the stimulating mitogen in the indicator system in that peripheral blood MNC from patients were not able to be induced by Con A to generate suppressor cells capable to suppressing subsequent Con A induced mitogenesis of autologous MNC ( $p < .025$  compared to controls).

We next utilized two other indicator systems different than Con A or PHA, which are predominately general T cell mitogens. Thus we examined both pokeweed mitogen which is a T cell dependent B cell activator as well as the allogeneic mixed leukocyte reaction in which foreign cell surface membrane antigens are the stimulating molecules. As can be seen in Table 2, after Con A pretreatment for 48 hours the suppressor activity of the malarious patients MNC, as assessed in using the PWM indicator system, was less than the activity of control MNC in this system, although the statistical significance ( $p < .06$ ) is not of the degree seen when the PHA or Con A indicator systems were used.

The allogeneic mixed leukocyte reaction is an antigen specific immunologic system in which T cells respond to LD determinants on the stimulating cells. As shown in Table 2, after incubation for 48 hours with Con A, normal controls suppressed the allogeneic mixed leukocyte reaction with a value of 58.5 ± 7.4% suppression. A similar level of suppression was found when Con A induced patients MNC were able to suppress autologous MNC responsiveness to allogeneic stimulator cells, 51.5 ± 26.8. Thus, there is no difference in the degree of suppression by the Con A induced patients' MNC compared with the controls' MNC in regard to autologous responder cells in the mixed leukocyte reaction.

Given the decreased number of circulating T cells in the peripheral blood of patients with acute malaria we isolated highly enriched T cells from the MNC of two malarious Thais and two healthy individual, incubated the T cells for 48 hours with Con A, and examined the level of suppressor cell activity generated by an equal number of T cells compared to unfractionated MNC in an autologous Con A indicator system. As shown in Table 3 enriched T cells from malarious patients exhibited higher suppression than did an equal number of unfractionated MNC. Furthermore, the degree of Con A induced suppression when enriched T cell from the malarious patients were used was equal to or greater than the degree of suppression exhibited when enriched T cell from the healthy individuals were used in the indicator system.

We have further assessed Con A suppressor cell activity in patients with malaria using allogeneic indicator cells because of difficulties in obtaining autologous indicator cells. This stems from the fact that naturally infected Thai patients with malaria are all out-patients in endemic areas of Thailand who come to an out-patient facility for diagnosis and treatment and subsequently

seldom come back for follow-up unless the treatment is ineffective or another complication ensues.

In the studies performed the ability of suppressor cells to be generated by incubation for 48 hours with Con A was assessed in indicator systems that measured the subsequent responsiveness of normal cells to the mitogenic lectins Con A, PHA and PWM, as well as the ability of normal cells to respond to allogeneic cell surface antigens in the mixed leukocyte reaction.

Figure 1 depicts the subsequent responsiveness of normal peripheral blood MNC to Con A after incubation with either normal controls' or malaria patients' MNC that had been preincubated for 48 hours with Con A. As can be seen, normal controls were induced to exhibit marked suppressor cell activity after Con A induction, with a mean suppression of  $43.8 \pm 6.0\%$  (mean  $\pm$  S.E.M.). However, when peripheral blood MNC from patients with malaria were examined, a markedly reduced degree of suppression of normal peripheral blood MNC was seen, with a mean suppression of only  $1.0 \pm 11.8\%$ . The differences in suppressor cell generation capacity (suppressor cells able to be generated in normal controls but not in malaria patients) were statistically significant ( $p < .01$ ).

We also found similar results using PHA as the stimulating mitogen in the indicator system in that peripheral blood MNC from patients were not able to be induced by Con A to generate suppressor cells capable of suppressing subsequent PHA induced mitogenesis of normal allogeneic peripheral blood MNC ( $p < .05$  compared to controls).

Although these experiments (Figure 1) indicate that patients with malaria have a decreased ability to generate suppressor cells after Con A stimulation, it should be noted that different normal allogeneic indicator cells were used for each experiment. Therefore, as shown in Figure 2, we examined six separate patients (four with *P. vivax* and two *P. falciparum*) using a single donor as a source of Con A stimulated indicator cells. The use of a single donor allows better control and stability with regard to the influence of possible indicator cell variability on the results of the previous experiment. As can be seen in Figure 2, after Con A treatment for 48 hours, six different individual normal controls exhibited good suppressor activity ( $36.8 \pm 6.2\%$ ) using one single individual's peripheral blood MNC as the Con A stimulated indicator cells. In contrast, peripheral blood MNC from the six patients not only showed no generation of suppressor cells after Con A stimulation but instead augmentation ( $14.3 \pm 7.2\%$ ) was seen, thus resulting in an overall difference of 51.1%, a result very similar to the data in Figure 1 and a difference which was statistically significant ( $p < .01$ ). Therefore, whether indicator cells from multiple individuals or from a single individual are used, Con A is able to induce much better suppressor activity from normal peripheral blood MNC than patient peripheral blood MNC when subsequent Con A stimulation of normal allogeneic peripheral blood mononuclear indicator cells is used to assess suppression.

We next turned our attention to utilization of two other indicator systems different than Con A or PHA. As can be seen in Figure 3, after Con A pre-treatment for 48 hours neither normal control nor patients peripheral blood MNC generated suppressor cells capable of significantly suppressing subsequent

PWM induced blastogenesis by normal allogeneic peripheral blood MNC. Thus, not surprisingly there was no significant difference between the normal control and patient populations. This decreased suppressor cell generation toward pokeweed mitogen induced mitogenesis, along with the PWM results in the autologous indicator system (Table 2) may signify that the suppressor activity being measured in Con A induction is associated principally with inhibition of T cell mitogen induced proliferation.

As shown in Figure 4, after incubation for 48 hours with Con A, normal controls suppressed the allogeneic mixed leukocyte reaction with a value of  $28.3 \pm 5.2\%$  suppression. However, when peripheral blood MNC from patients infected with malaria were examined, not only was suppression not observed, but instead marked augmentation was seen in that the mixed leukocyte reaction was greater than normal in over half of the combinations examined and the final augmentation was  $63.2 \pm 29.0\%$ . Once again the difference between normal controls and patients was large (91.5%) and was statistically significant ( $p < .01$ ).

A critical question is whether the defect in suppressor cell generation capability seen in patients with malaria, is related to the activity of the disease process. Three individuals who developed malaria were studied serially. The clinical data with regard to these patients is given in Table 4 and the results of the immunologic studies shown in Figure 5. Two indicator systems were examined with regard to the ability of Con A to induce suppressor cell activity in peripheral blood MNC. That is, control and patient cells after Con A pretreatment were assessed for their ability to suppress the allogeneic mixed leukocyte reaction and subsequent Con A induced mitogenic responsiveness of normal allogeneic cells. These two assay systems were chosen, because patients infected with malaria consistently showed decreased suppressor generation in these allogeneic assays (Figure 1 and Figure 4). As shown in Figure 5, when the patients were actively infected and sick, none exhibited any degree of suppressor cell activity after Con A pretreatment and MNC from patient 3 caused marked augmentation of subsequent Con A and allogeneic MLR cultures. However, seven days after treatment had begun, despite the loss of Con A suppressor generation capability prior to treatment, MNC from all three patients had completely recovered normal suppressor cell generation capabilities. Furthermore, MNC from patient 3, when tested three weeks after therapy, maintained normal suppressor cell generation capabilities. In contrast, both patients 1 and 2 had relapses of their malaria infection and both once again exhibited loss of Con A induced suppressor cell generation coinciding with the return of disease activity and positive smears. This loss of suppressor cell activity continued for both of the patient while they were in relapse.

## DISCUSSION :

To accurately characterize the effect of malaria on the immune response, examination of naturally infected humans is essential so that immunologic alterations in the individuals who will eventually receive a malaria vaccine can be delineated. In our previous studies, we have found that a major immunologic alteration in patients with malaria is a decrease in the percentage and concentration of T lymphocytes (Wells et al. 1979).

In order to understand the significance of the alteration of lymphocyte subpopulations in malarious patients we have begun to assess the functional capabilities of lymphocyte subpopulations in a variety of cellular assays. In the present study Con A stimulated patients' lymphocytes were used to investigate whether the cells exhibit an inhibitory effect in a non-specific indicator system of autologous or allogeneic responder lymphocytes. By using the allogeneic assay system it was possible to avoid using malarious responder cells which besides being difficult to obtain in our field studies might potentially respond differently than normal responder cells in the assay. Horowitz et al. (1977) and Buschard, Madsbad & Rygaard (1980) have shown in separate Con A suppressor cell activity studies of patients with system lupus erythematosus (SLE) and insulin dependent diabetes mellitus, respectively, that the results obtained did not depend on the type of cell (autologous or allogeneic) used as responders. Furthermore, in our control cultures incubation of test cells and responder cells without mitogenic lectin resulted in little allogeneic stimulation (< 7.9%) compared to that exerted by mitogenic lectin.

The results of our present study indicate that functionally this decrease in T lymphocytes in part manifests itself as a decrease in suppressor cell generation capability after Con A stimulation. It should be noted that this is not the only defect associated with loss of T cells in malaria patients. Indeed, we have previously demonstrated that peripheral blood MNC from patients with malaria have decreased responsiveness in the allogeneic mixed leukocyte reaction (MacDermott et al. 1980). Furthermore, sera from patients with active malaria will induce suppression of normal peripheral blood MNC responsiveness to the T cell mitogenic lectins PHA and Con A (MacDermott et al. 1980). Finally, in studies recently completed we have found that mitogen induced cellular cytotoxicity, which is predominantly a T cell function, is depressed in Thai adults with both *P. falciparum* and *P. vivax* (Gilbreath et al. submitting for publication). Thus, the present studies, in conjunction with other studies we have performed, indicate that both physically and functionally a major defect in T cells occurs in patients with malaria and serum factors or antibodies are capable of further augmenting these defects.

Although it is not always correct to extrapolate from *in vitro* findings to postulate *in vivo* biologic events, there are several points which can be made with regard to our current studies and the immune response of patients with malaria. Firstly, increased levels of autoantibodies 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 (Shaper et al. 1968). Thus, it is possible that the lack of suppressor cell activity noted in the present study which occurs during malaria infection might lead to lack of normal regulatory control of antibodies in general and IgM in particular with perhaps specific autoantibodies resulting. Furthermore, other autoantibodies which have been found include antibodies directed against peripheral blood MNC (Wells et al. 1980) and red blood cells (McGregor 1972). It is possible that the antibodies we have found to be directed against peripheral blood MNC might be directed specifically against suppressor T cells and, by causing their deletion result in both the lack of suppression seen in the present study as well as increased autoantibody production. However, it is also possible that lack of suppressor cell activity

initiates the production of antilymphocyte antibodies. Finally, antigen-antibody complexes have been shown to cause nephrotic syndrome in young children with malaria and lack of suppressor cell regulation leading to heightened antibody response to the malaria parasite could ultimately result in increased deposition of antigen-antibody complexes in the kidney (McGregor 1971, 1972).

The question which next arises is the reason for decreased suppressor cell activity in patients naturally infected with malaria. We feel that the changes are most likely a direct result of the malaria infection and thus either could represent part of the normal host immune response (Phillips et al. 1970, McGregor 1971, Hamburger & Kreier 1975) to parasitic infection or alternatively could represent an adverse effect of the parasite on the host immune system (Jayawardena et al. 1975). The actual decrease in functional activity could be due to physical loss of the suppressor cells, perhaps due to destruction by anti-lymphocyte antibodies or cytotoxic substances released during the parasitic infestation. Alternatively, the suppressor cells may be present in normal numbers but rendered inactive by interference with normal metabolic function, although the results of the T-cell enrichment experiments argue against this. Finally, the third possibility is that the suppressor cells have specifically undergone a transient relocation from the peripheral blood by altered migration into a different lymphocyte pool, such as the spleen (Wyler, Miller & Schmidt 1977) or lymph nodes.

Although we are measuring alterations in suppressor cell generation, the relevant functional changes with regard to malaria infection may be totally different. That is, cells within the same subclass as suppressor cells also are effectors of T cell mediated cell mediated lympholysis. Thus, if T or K cells (Brown & Smalley 1980) are potentially important in the normal immune response to foreign invaders by lysis and destruction of potentially harmful organisms, one critical aspect of successful parasite infection may include neutralization or removal of the T or K cell mediated defense mechanisms by the parasite as suggested by the studies of Jayawardena et al. (1975). Alternatively, the T cell mediated defense mechanisms through a sensitization and direct cytotoxic effector cell system could be functioning correctly in the infected host. However, the ongoing T cell mediated killing may take place in other organs than the peripheral blood and therefore the specific subclass of T cells which is carrying out the cytotoxic effector function and is destroying the malaria parasite, has perhaps transiently relocated into a different lymphocyte pool, such as the spleen (Wyler et al. 1977).

In the present studies, the loss of T cell function occurred during active disease involvement and not during drug induced remission in the three patients who were followed serially. This implies that the abnormalities of T cell suppression induced by infection with malaria is related to disease activity, are not permanent, and are reversible. Furthermore, in two patients who were successfully treated, there was a return to normal of suppressor cell functional capabilities, but subsequently a loss of Con A inducible suppressor cell activity simultaneously with relapse of disease. This indicates that normal suppressor cell induction capacity can precede abnormalities induced by subsequent malaria infection.

When pokeweed mitogen was used to stimulate the indicator MNC, there was little (autologous) or no difference (allogeneic) between normals and patients with regard to Con A induced suppressor cell function, while significant differences were seen with Con A, PHA, and allogeneic cell surface antigen stimulation (using the allogeneic system) of indicator MNC. Likewise, in our previous study examining the effects of serum on mitogen induced blastogenesis (MacDermott et al. 1980), there was no effect on PWM induced blastogenesis while marked suppression was seen with the mitogenic lectins Con A and PHA. Although these findings are not necessarily related, it may be indicative of greater alterations in T-T cell interactions as opposed to T-B cell interactions. Finally, it is also important to recognize that other possible cell interactions may account for the present results. That is, macrophages, as well as T cells, could be functioning abnormally since macrophages are necessary for normal T cell function and can also serve as suppressor cells themselves. Likewise, it needs to be re-emphasized that the present data holds only for *in vitro* cultures of peripheral blood MNC and although one would like to be able to extrapolate to *in vivo* situations this needs to be done with caution since T-T cell or macrophage-T cell interactions could be entirely normal in other lymphoid tissues such as the spleen or lymph nodes.

Finally, one of the intriguing aspects of the present study is the relation of the alterations seen in malaria with other diseases in which deficiency of suppressor cell generation has been noted. There are numerous illnesses in which Con A generation of suppressor cells has been shown to be deficient, of which SLE is the principle example (Strelkauskas et al. 1978; Sakane et al. 1979). The data in the present study not only adds to the list of diseases in which alterations of Con A induced suppressor cell activity occur but also points out that such alterations may not be primary to an autoimmune disease process. That is, our primary interpretation of the data in the present study is that the changes arise secondary to the parasitic infection as part of the host immune response and most simply might be explained by a migration of the cells to other parts of the immune system. Therefore, it is of interest that the abnormalities seen in our series of studies on malaria are essentially identical to those seen in SLE. This is, in both malaria and SLE there is a loss of T cells in general, a loss of suppressor cell activity in particular, the presence of antilymphocyte antibodies and multiple serum induced suppressor effects. The major implication of the similar changes seen in malaria and SLE is that it leads to the general hypothesis that challenge to the host with a parasite, virus, or other agent leads to alterations in the regulatory capabilities of the immune system which subsequently lead to autoimmune manifestations in some individuals or a primary autoimmune disease in others.

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Table 1. Autologous suppressor activity of Con A-activated mononuclear cells (MNC) for the response of fresh MNC to PHA.

Exp. No.	Con A activation of MNC in first culture period	$(^3\text{H})$ TdR Incorporation in response to PHA					
		Patient*			Control		
		CPM $^3\text{H}$ -thymidine	(S.I.)**	% suppression***	CPM $^3\text{H}$ -thymidine	(S.I.)	% suppression
1	-	1997 ± 572	(11.9)	202 (AUG) ++	7365 ± 879	(26.9)	59.1
	+	6051 ± 564	(35.9)		3709 ± 663	(11.0)	
2	-	8029 ± 648	(33.6)	38.7	15050 ± 2076	(40.0)	57.2
	+	13725 ± 945	(20.6)		14067 ± 698	(17.1)	
3	-	7709 ± 269	(25.4)	4.8	8965 ± 453	(29.3)	47.8
	+	8166 ± 593	(24.2)		8477 ± 751	(15.3)	
4	-	12633 ± 273	(49.8)	45.4 (AUG)	5039 ± 465	(27.3)	59.0
	+	15559 ± 845	(72.4)		4044 ± 110	(11.2)	
5	-	2534 ± 179	(27.2)	48.5	4204 ± 137	(51.8)	89.8
	+	2450 ± 311	(14.0)		3566 ± 475	( 5.3)	

\* All experiments were performed with MNC from patients with *Plasmodium falciparum* infection.

\*\* S.E. = CPM in presence of PHA/background CPM in absence of PHA.

\*\*\* Percent suppression computed as described in *Materials and Methods*.

† Values are the mean ± S.E. of CPM for triplicate cultures.

†† Augmentation of responsiveness.

††† Mean ± S.E. of suppression computed by setting augmentation values equal to zero. Statistically significant difference compared to control values ( $p < .005$ ) using two-tailed paired t-test.

Table 2. Autologous Con A suppressor activity of malarious patients or control mononuclear cell populations in mitogenic lectin induced and one way mixed lymphocyte reaction assays.

Exp. of MNC in first culture period	Con A activation of MNC in first culture period			Patient*			Con A activation of MNC in first culture			Control				
	Con A	PWM	MLR**	Con A	PWM	MLR**	Con A	PWM	MLR	Con A	PWM	MLR		
	S.I.*** % supp. +	S.I. % supp.	S.I. % supp.	S.I. % supp.	S.I. % supp.	S.I. % supp.	S.I. % supp.	S.I. % supp.	S.I. % supp.	S.I. % supp.	S.I. % supp.	S.I. % supp.		
1	-	2.7	77.8 (AUG)	8.4	51.2	2.2	100 (AUG)	-	26.5	82.3	28.4	47.9	2.0	45.0
	+	4.8		4.1		14.1		+	4.7		14.8		1.1	
2	-	34.4	17.7	90.9	44.8	N.D.		-	15.1	58.3	46.0	54.1	N.D.	
	+	28.3		50.2				+	6.3		21.1			
3	-	8.9	23.6	51.3	5.7	10.1		-	23.0	77.8	47.7	39.8	2.5	60.0
	+	6.8		48.4		1.0		+	5.1		28.7		1.0	
4	-	13.2	60.6 (AUG)	64.2	42.7 (AUG)	N.D.		-	12.3	52.0	28.7	47.0	N.D.	
	+	21.2		91.6				+	5.9		15.2			
5	-	13.7	67.9	41.9	52.0	2.8	64.3	-	40.9	89.2	86.5	86.1	1.7	70.6
	+	4.4		20.1		1.0		+	4.4		12.0		0.5	
Mean ± S.E.		21.8±12.4 <sup>++</sup>		30.7±11.5 <sup>+++</sup>		51.5±26.8			71.9±7.2		54.9±8.1		58.5±7.4	

\*Patient MNC were from the same individuals as the MNC used for the experiment represented in Table 1.

\*\* Mean ± S.E. for spontaneous CPM in mitomycin C treated stimulator cell population was (168.0 ± 38.0); mean ± S.E. for the same cells incubated with mitogen was found to be (170.0 ± 42.0). Mean ± S.E. for spontaneous CPM of patient and control responder cells were (150.8 ± 25.0) and (212.5 ± 47.5) respectively.

\*\*\* S.I. - CPM in presence of mitogen or stimulator cells/background CPM in absence of mitogen or stimulator cells.

+ Percent suppression computed as described in *Materials and Methods*. Mean ± S.E. computed for each group by setting augmentation values equal to zero.

++ Statistical significant difference compared to controls (p < .025) using 2 tailed paired t-test.

+++ Statistical significant difference compared to control values (p < .06) using a two tailed t-test.

Table 3. Level of suppression in mitogen stimulated responder cell cultures incubated with an equal number of malarious patients or control Con A activated autologous T-cells or mononuclear cells.

Exp. No.	Source of second culture responder cells	Number of autologous mitomycin C treated first culture cells added to second culture*	Percent ERFC**	Con A activation of MNC in first culture period	S.I.***	% suppression <sup>†</sup>
1	Patient	(4 x 10 <sup>4</sup> MNC <sup>††</sup> )	(56%)	-	6.5	20.0
	Patient	(4 x 10 <sup>4</sup> ERFC <sup>†††</sup> )	(>95%)	+	5.2	
	Control	(4 x 10 <sup>4</sup> MNC)	(62%)	-	2.7	82.0
	Control	(4 x 10 <sup>4</sup> ERFC)	(>95%)	+	15.1	
2	Patient	(2 x 10 <sup>5</sup> MNC)	(34%)	-	5.9	39.0
	Patient	(2 x 10 <sup>5</sup> ERFC)	(95%)	+	3.6	
	Control	(2 x 10 <sup>5</sup> MNC)	(42%)	-	3.3	42.5
	Control	(2 x 10 <sup>5</sup> ERFC)	(95%)	+	1.9	
	Patient	(2 x 10 <sup>5</sup> MNC)	(95%)	-	10.4	11.5
	Patient	(2 x 10 <sup>5</sup> ERFC)	(95%)	+	9.2	
	Control	(2 x 10 <sup>5</sup> MNC)	(42%)	-	19.8	25.8
	Control	(2 x 10 <sup>5</sup> ERFC)	(95%)	+	14.7	
					11.2	22.3
					8.7	
					19.6	29.1
					13.9	

\*These cells were introduced into freshly prepared autologous responder cells (1 x 10<sup>5</sup> per well) in the assay culture which were stimulated with Con A.

\*\*Percent of E-rosette forming cells (T-cells) in respective first culture.

\*\*\*S.I. = CPM in presence of Con A/background CPM in absence of Con A.

†Percent suppression computed as described in *Materials and Methods*.

†† Mononuclear cells.

††† Enriched populations of ERFC was obtained as described in *Materials and Methods*.

Table 4. Clinical profile of three patients with naturally-acquired *P. vivax* malaria who were studied serially (Figure 5).

Patient	Age	Race	Parasitemia	Prophylaxis	Therapy	Outcome
1	45	Thai	4/10,000 rbc	Yes*	Chloroquine** Primaquine	R <sup>+</sup>
2	47	Thai	Positive <sup>x</sup>	Yes*	Chloroquine** Primaquine	R <sup>+</sup>
3	42	Caucasian	Positive <sup>x</sup>	Yes*	Chloroquine** Primaquine	Cured

\*2 doses of Fansidar (2 tablets every 14 days) prior to patient parasitemia.

\*\*Standard dose of 1500 mg. chloroquine base followed by 14 day course of primaquine.

<sup>+</sup>Relapse as noted in Figure 5.

<sup>++</sup>G6PD deficient.

<sup>x</sup>Parasitemia verified using Giemsa stained thick and thin smear.

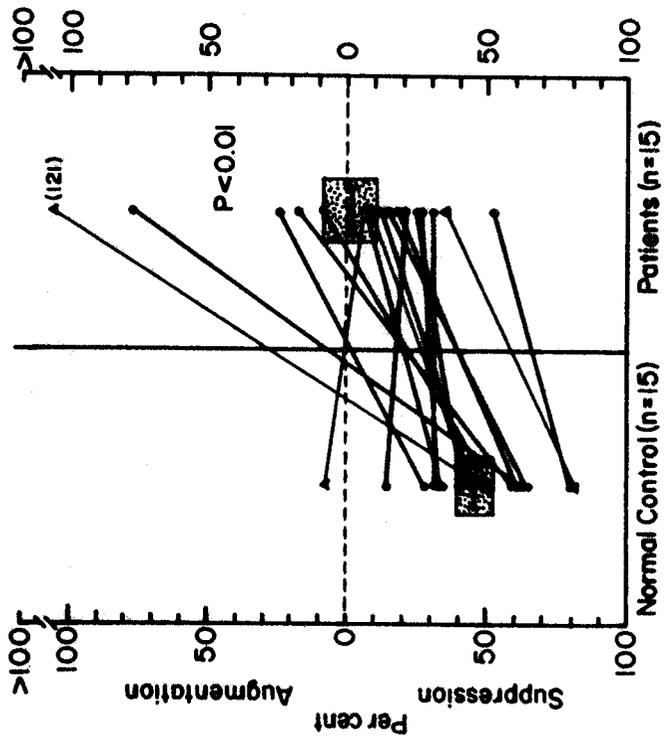


Fig. 1. Effect of Con A-pretreated cells from malarious Thai patients or normal controls on responses to Con A by allogeneic cells from healthy donors. Mean values are indicated by horizontal bars, (▲) *P. vivax* patients; (●) *P. falciparum* patients, shaded boxes represent S.E. M.

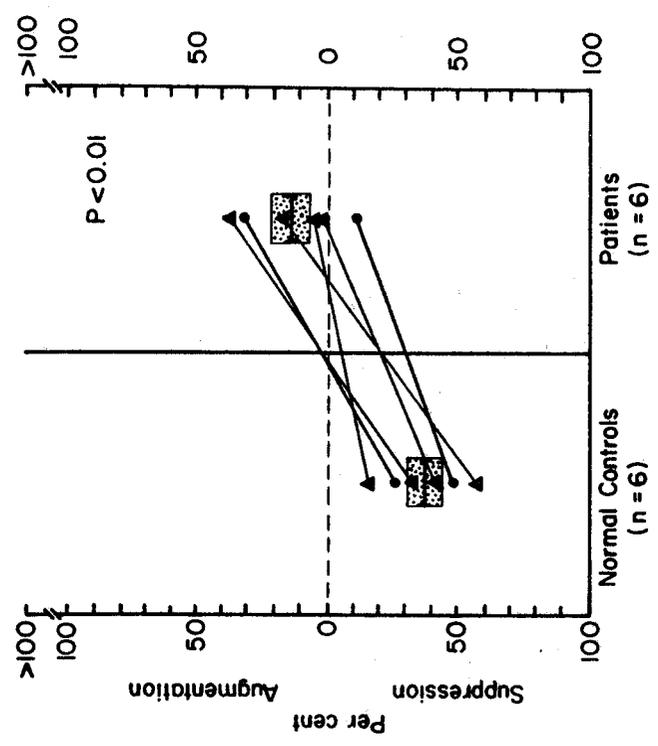


FIG. 2. Effect of Con A-pretreated cells from patients or normal controls on responses to Con A by allogeneic responder cells from a single donor source. Symbols as in Fig. 1.

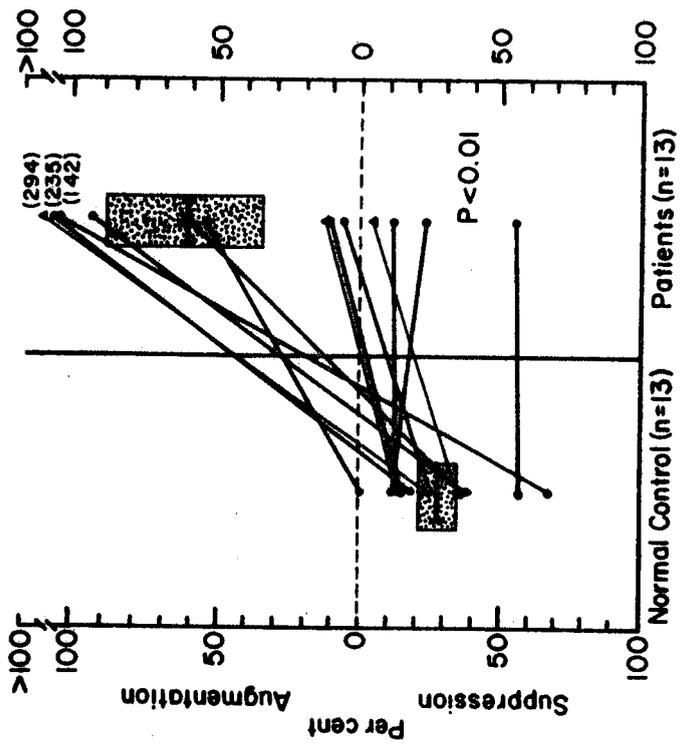


Fig. 4. Effect of Con A-pretreated cells from malarious Thai patients or normal controls on the one way mixed lymphocyte reaction by allogeneic cells from normal donors. Symbols as in Fig. 1.

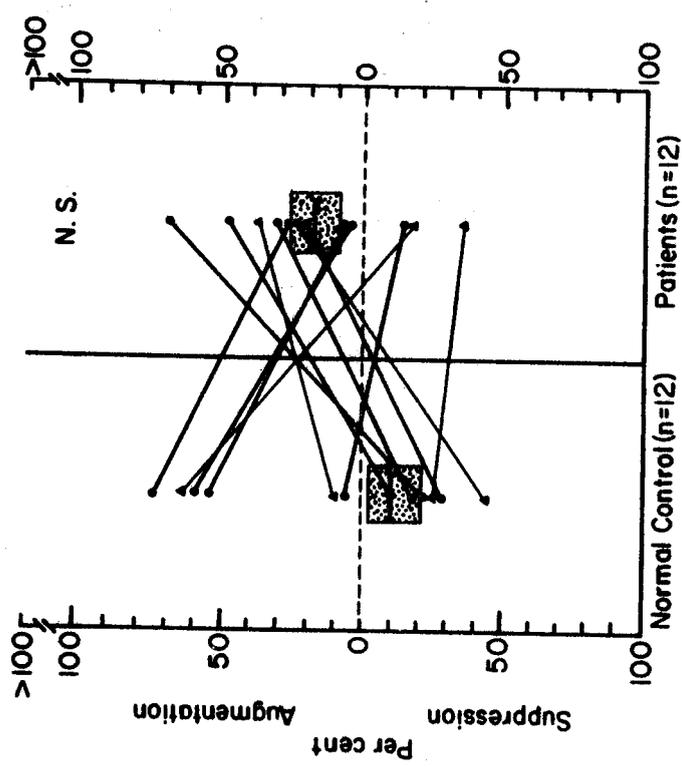


Fig. 3. Effect of Con A-pretreated cells from malarious Thai patients or normal controls on responses to PWM by allogeneic cells from healthy donors. Symbols as in Fig. 1.

Fig. 5. Suppressor activity of malarious patient's Con A-pretreated MNC at different times during treatment.

