

DEFICIENT SPONTANEOUS CELL MEDIATED CYTOTOXICITY AND
LECTIN-INDUCED CELLULAR CYTOTOXICITY BY PERIPHERAL
BLOOD MONONUCLEAR CELLS FROM THAI ADULTS
NATURALLY INFECTED WITH MALARIA

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OBJECTIVE : To investigate non-specific cytotoxic effector cell capabilities of mononuclear cells from malarious Thais.

BACKGROUND : A detailed understanding of the host immune system's response to malaria infection will result in clarification of the normal human response to parasitic infection as well as elucidation of the ways in which a parasite modifies the host immune response in order to avoid neutralization or destruction. We have therefore undertaken a series of experiments designed to determine the immunologic alterations which occur during parasitic infection with *P. falciparum* and *P. vivax* in naturally infected Thai adults. Our studies to date have demonstrated that patients with malaria have a true decrease in circulating T cells but no real change in Null or B cell numbers (Wells et al., 1979); antilymphocyte antibodies in their sera (Wells et al., 1980) a decrease in suppressor T cell generation capability (Gilbreath et al., submitted for publication); and serum factors capable of inhibiting normal lymphocyte blastogenesis (MacDermott et al., 1980c). Because the studies which we have carried out to date have not assessed cellular effector functions, we chose in the present study to begin examination of peripheral blood (PB) mononuclear cell (MNC) mediated cytotoxicity, using MNC from Thai adults naturally infected with *P. falciparum* and *P. vivax*. The results of the present experiments indicate that patients with malaria have defective T cell and NK cell cytotoxic capability in some systems but do not exhibit defective K cell function. These abnormalities may be induced by the malaria parasite in order to allow continued replication.

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MATERIALS AND METHODS :

Patients : Serum and peripheral blood mononuclear cells (MNC) were obtained from 52 male patients with naturally acquired *P. falciparum* (33 patients) or *P. vivax* (19 patients) malaria. The patients were diagnosed by the medical staff of the malaria Eradication Center (MEC), Phrabuddabat, Thailand. All the patients had low levels of parasitemia ($< 0.05\%$) (mean % (\bar{x}) parasitemia \pm standard error (S.E.M.): 0.029 ± 0.004), (age ($\bar{x} \pm$ S.E.M.) 25.6 ± 1.9) and denied being on any medication at the time of testing. All patients were subsequently treated as "out patients" by the MEC medical staff. An age-and-sex matched healthy Thai volunteer who had not previously had malaria served as a control donor for each patient and was bled at the same time of day so that the cells could be tested simultaneously with the patients' cells. Thus different, normal, fresh controls were used concurrently in all assays. Concurrent matched controls are necessary because of day to day variations in the assays. These variations can be appreciated by comparing the control values in the different figures (i.e., Figure 2a vs 2b, Figure 3a vs 3b, and Figure 4a vs 4b), and may be due to differences in the number of patients examined, the cycle or condition of the target cells, or seasonal variations in effector cell function.

Isolation and "Fractionation" of Peripheral Blood (PB)

Mononuclear Cells : Effector cells were obtained from 15 ml heparinized PB (10 units heparin/ml) and isolated using Ficoll-Hypaque density gradient centrifugation (Boyum, 1968) as previously described (Wells et al., 1979). Briefly, MNC were washed three times and resuspended in final medium (RPMI 1640 (GIBCO) containing 2 mM glutamine, 25 mM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid buffer, 50 U of penicillin, 50 μ g of streptomycin per ml, and 20% heat-inactivated fetal bovine serum (FBS))

Some MNC preparations were "fractionated" into adherent and nonadherent cell populations by passing the MNC preparation over a Sephadex G-10 column as previously described (MacDermott and Stacey, 1981). Cells were resuspended in final medium, counted, and viability determined using the eosin dye inclusion technique.

In some cases a small amount of nonheparinized blood was obtained from the patients for use in serum regulatory studies. After permitting the blood to clot at room temperature serum was sterilely collected, refrigerated, and used within 24 hours. Additional serum samples, previously screened for lymphocytotoxic activity as described by Wells et al., (1980), and subsequently frozen at -20°C were also used in some studies to test for serum regulatory factors.

Preparation of Antisera : Anti-Chang cell antibody was prepared by injecting adult male rabbits (weighing 3 kg each) with 4×10^6 Chang liver cells every two weeks for three months. The rabbits were bled two weeks later and serum was collected from the clotted blood. Commercial rabbit anti-chicken red blood cell antibody was obtained from Cappel Laboratories (Lot #11871, Cochranville, PA). Before use, all antisera were heat-inactivated for 60 minutes at 56°C .

Preparation of ^{51}Cr Labelled Target Cells : The human myeloid cell line, K562, was maintained in suspension culture. Chang liver cell line cells (Microbiological Associates, Bethesda, MD) were maintained in minimal essential media (MEM) while fresh CRBC were obtained daily from our lab animal facility. Fresh allogeneic human erythrocytes (HRBC) were obtained daily from normal human donors. Labelled target cells were prepared by washing the cells in assay media three times and resuspending the cell line cells or RBC at 1×10^6 cells/ml or 4×10^6 cells/ml, respectively. Then 0.1 ml of each cell suspension was incubated for one hour at 37°C with 0.1 ml chromium isotope (Sodium $\text{Na}_2(^{51}\text{Cr})\text{O}_4$ in saline, sp act 425 mc/mg, New England Nuclear, Boston, MA). The target cells were washed three times in media and resuspended to a final concentration of 1×10^6 Chang cells/ml; 1×10^5 K562 cells/ml; 1×10^7 CRBC/ml; or 5×10^5 HRBC/ml.

Cytotoxicity Assays : To carry out K562 SCMC, dilutions of unfractionated, nonadherent or adherent effector cells (0.1 ml) and ^{51}Cr -labelled K562 target cells (0.1 ml) were incubated for four hours at 37°C in a 5% CO_2 , 95% air, humid environment, as described previously by MacDermott et al (1980a, 1980b). The ADCC, LICC, and SCMC assays with Chang or CRBC targets were performed as previously described (MacDermott et al, 1980a, 1980b). In brief, fifty microliters of antibody, PHA, or medium were added to the wells containing 0.05 ml of the labelled CRBC or Chang target cells. The optimal dilution of anti-CRBC serum, 10^{-5} ; anti-Chang serum, 1/3000; or PHA, 1 $\mu\text{g}/\text{ml}$, were determined in preliminary experiments and were used throughout the study. Fifty microliters of unfractionated or fractionated (macrophage depleted cells or macrophages) MNC populations were then added (at a concentration ranging from 1 to 25×10^6 cells/ml) to the wells. The Chang and CRBC cytotoxicity assays were incubated for 18 hours prior to harvesting and counting. The HRBC, LICC assays were performed as previously described (MacDermott et al., 1976, 1980a, 1980b) and modified as follows : dilution of unfractionated, nonadherent or adherent effector cells (0.05 ml) and ^{51}Cr -labelled HRBC (0.05 ml) were incubated with WGA, 8 $\mu\text{g}/\text{ml}$, in round bottom microtiter plates for 18 hours at 37°C in 5% CO_2 . The WGA concentration used throughout the study was determined from preliminary experiments and was not cytotoxic to the HRBC in the absence of effector cells.

All cytotoxicity tests were performed in triplicate using 96 well round bottom microtiter plates (Flow Laboratories, Rockville, MD). Maximal ^{51}Cr release was determined by adding 5% Triton X-100 to incubation mixtures containing labelled target cells. The spontaneous release of isotope by the labelled target cells were essentially the same in cultures with MNC as in cultures without MNC. After the incubation period the culture mixtures were harvested using a Titertek harvester (Flow Laboratories) and the amount of radioactivity released from the cells were determined using a Packard Model 5835 gamma counter. Data were calculated for the assays by the following formula : $(E-S/\text{max}-S) \times 100 = ^{51}\text{Cr}$ specific release, where E = c.p.m. released from target cells plus effector cells; S = c.p.m. released spontaneously from target cells; and max = c.p.m. released after addition of 0.5 ml 5% Triton X-100.

Serum Regulatory Studies : A series of experiments were carried out to assess the effect of malarious patients' serum on the ability of MNC to function as effector cells in the SCMC, ADCC, and LICC assays.

In the experiments examining SCMC and LICC, the inhibitory effect of autologous serum on the patients' MNC was assessed as previously described (Silverman and Cathcart, 1980). Briefly, the MNC's, SCMC and LICC activity was studied before and after overnight incubation in serum free medium. After overnight incubation 5×10^6 MNC per ml were incubated for two hours at 37°C with an equal volume of 20% (v/v) heat-inactivated autologous or allogeneic serum or media. Pooled serum from healthy male donors or FBS served as serum controls in these experiments. After washing the MNC one time and diluting the MNC to a 25:1 effector to target cell ratio, the assays were performed as described above.

In the experiments examining ADCC, a modification (Meyer and Descamps, 1979) of the ADCC inhibition assay described by Perlmann and Perlmann (1970) was used. Human effector lymphocytes from healthy donors were preincubated for one hour at 37°C with 20% (v/v) of either heat-inactivated patient sera, normal human control sera, or a pool of normal human sera prior to adding the suspension to the target cells. The pool of normal human sera was included in each test as a control for normal serum since serum heat inactivation, which is necessary for eliminating the participation of complement in ADCC models, can provide slight IgG aggregation leading to a slight, but constant reduction of ADCC.

In a second set of experiments, an equal volume of malarious patients' serum, previously tested for the presence of cold-reactive (15°C) lymphocytotoxic activity (LCA) as described by Wells, et al., (1980) was incubated with MNC from healthy donors for two hours at 37°C prior to testing the effector MNC in the SCMC and LICC assays. Sera grouped into those sera having high LCA (> 40%) and those sera with low LCA (< 40%) were used to determine if any sera regulatory factors effective against SCMC or LICC effector MNC were present in malarious patients' serum and if the regulatory factors were associated with the presence of serum LCA. Cells were then mixed with labelled target cells at the desired effector to target cell ratios.

The following equations were used to determine the level of inhibition :

$$(a) \quad \% \text{ } ^{51}\text{Cr release} = \frac{\text{supernatant c.p.m.}}{\text{supernatant c.p.m.} + \text{pellet c.p.m.}} \times 100$$

(b) Specific cytotoxicity index according to Brunner's formula :

$$\text{CI} = \frac{\% \text{ } ^{51}\text{Cr test release} - \% \text{ } ^{51}\text{Cr control release}}{\% \text{ } ^{51}\text{Cr max. release} - \% \text{ } ^{51}\text{Cr control release}}$$

Where, according to treatment of (^{51}Cr) labelled target cells, test release is the combination of test sera with rabbit anti-Chang serum or rabbit anti-CRBC serum, control release that without test serum and maximum release that with 5% Triton X-100.

Statistical Significance : Statistical significance of the results was assayed by the student's t-test and paired-sample test.

RESULTS :

SCMC, ADCC, and LICC with Cell Line Cells as Targets : We first examined spontaneous cell mediated cytotoxicity (SCMC), in which the effectors are "natural killer" (NK) cells. SCMC against K562 targets by peripheral blood MNC from malarious Thai adults was significantly lower ($p < 0.05$) than with MNC from healthy controls at 5:1, 25:1, and 50:1 effector to target cell ratios (Figure 1). Although patients with malaria had deficient NK cell activity using K562 cell line cells as targets, when Chang cell line cells were used as targets, normal levels of SCMC were seen (Figure 2). Thus, the NK cell deficiency is not total, but rather only present with certain targets. Furthermore, as shown in Figure 3 and Table 1, antibody dependent cellular cytotoxicity (ADCC), a killer (K) cell mediated event, is unchanged in peripheral blood MNC from malaria patients using Chang cell line cells as targets. However, use of the lectin PHA to induce killing of Chang cell line targets (Table 1) revealed that malaria patients do exhibit statistically significant decreased LICC compared to normal controls using 50:1 and 25:1 effector to target cell ratios.

ADCC and LICC with Red Blood Cells as Targets : We next utilized a different target cell type (RBC's) in the assay systems, in order to begin studies using a type of cell which could potentially be of greater relevance in the study of malaria. Our studies examined the ability of patient peripheral blood MNC to lyse CRBC's in an ADCC assay system and HRBC's in an LICC assay system. The mean spontaneous release of isotope was 3.9% 0.6% for the CRBC targets and maximum isotope release ranged from 70% to 89%. Figure 4 shows the mean percent ADCC when MNC from control subjects or Thai adults with acute (a) *P. vivax* or (b) *P. falciparum* malaria were tested against CRBC target cells. No statistically significant difference was seen between control and patient ADCC at any of the effector to target cell ratios when unfractionated MNC, macrophage depleted lymphocytes, or macrophages were used as effector cells. MNC did not cause cytotoxicity in the absence of rabbit anti-CRBC antibody. Thus normal ADCC activity against CRBC was seen, similar to the findings above (Figure 3 and Table 1) for ADCC with Chang cell line cells as targets. When LICC was examined using wheat germ agglutinin (WGA) to induce killing of allogeneic human red blood cell targets, significantly decreased LICC ($p < 0.05$) was seen with patient MNC in comparison to control MNC in experiments performed at a 25:1 effector to target cell ratio (Figure 5).

Relationship of Decreased K562 SCMC and WGA induce LICC of HRBC and Effect of Macrophage Depletion : A comparison of each individual patient's SCMC (K562) and LICC (WGA-HRBC) responses indicated that a decreased killing capability in one of the assays was generally accompanied by a low response in the other assay (Figure 6). Six of nine patients had lower responses in both the SCMC and LICC assays than their respective age - matched controls (Figure 6). Therefore the majority of patients exhibiting a cytotoxic effector cell defect were deficient in more than one assay system. Since we examined only unseparated peripheral blood mononuclear cells, the next question which arose was whether the deficiency of cytotoxic effector cell capability existed primarily in the patient's lymphocytes, macrophages, or both cell subpopulations. As can be seen in Figure 7, defective LICC (WGA-HRBC) capacity existed in both the non-adherent MNC (lymphocyte) population as well as the adherent

MNC (monocyte-macrophage) population. In contrast, defective SCMC (K562) capacity existed only in the nonadherent MNC (lymphocyte) population, with the adherent MNC (monocyte-macrophage) population not exhibiting much killing with either control or patient MNC.

Effect of Patient Sera on ADCC, SCMC, or LICC Assays : The next question which was approached, was whether sera from patients with malaria could induce a defect in ADCC, SCMC, or LICC, using normal peripheral blood MNC as the effector cells. That is, in the assay systems in which patient MNC functioned normally, it could be hypothesized that removing the patient's MNC from their sera resulted in loss of inhibition. Furthermore, in the assay systems in which patient MNC exhibited decreased cytotoxic capability, serum factors might potentially account for the deficit.

Using a modification of the ADCC inhibition assay we attempted to demonstrate the presence of inhibitory factors in the serum of patients with *P. vivax* and *P. falciparum* malaria. MNC from healthy donors were incubated with sera from 14 individual patients (1 *P. vivax*, 13 *P. falciparum*) prior to use in the CRBC, ADCC assay, or from 3-9 individual patients (1 *P. vivax*, 8 *P. falciparum*) prior to use in the Chang ADCC assay. Although CRBC, ADCC was slightly decreased and Chang ADCC slightly increased in the presence of patient sera, no statistically significant difference in ADCC was seen in either the CRBC or Chang assay systems, when MNC incubated with patient or healthy donor serum was tested at three different effector target cell ratios (Table 2).

The SCMC and LICC response of MNC from malarious patients did not increase when the MNC were incubated overnight in serum-free medium at 37°C (Table 3). In fact, the SCMC response of three patients and three control MNC were consistently lower after overnight incubation. Incubation of the MNC with autologous serum had little effect on the MNC, SCMC response (Table 3).

Two malarious patients and two healthy control donors' MNC populations were also studied to determine the effect of overnight incubation in serum free medium on the LICC response (Table 3). LICC of the MNC incubated overnight was lower than LICC of fresh isolated MNC. Incubation of the MNC with autologous serum had little effect on LICC although malarious patients' serum tended to slightly decrease the LICC of MNC obtained from the healthy control donors (Table 3).

Finally, experiments in which nonadherent MNC from healthy donors were incubated for two hours with either malarious patients' serum that was known to contain cold-reactive lymphocytotoxic antibodies, autologous serum, or pooled control serum prior to testing the MNC in the SCMC and LICC assays, failed to demonstrate any SCMC or LICC inhibitory factors in malarious patients' serum (Table 4).

DISCUSSION :

We have undertaken in the present and previous studies to examine the immunological capabilities of naturally infected Thai adults who have *P. falciparum* and *P. vivax* malaria. The naturally infected host presents a unique and important opportunity to examine the mechanisms by which the malaria parasite evades recognition and destruction. In human malaria this is particularly intriguing since the naturally occurring infection can last up to two to ten years. Our studies suggest that due to the infection with the malaria parasite, a number of immunologic changes occur which may prevent the host from mounting an effective immune response against the *P. falciparum* or *P. vivax* organisms. In previous studies, the changes we have observed include a loss of circulating T cells (Wells et al., 1979), a loss of functional Con A inducible T suppressor cells (Gilbreath et al., submitted for publication), anti-lymphocyte antibodies (Wells et al., 1980), and serum suppressor factors (MacDermott et al., 1980c). However, our previous studies did not examine mononuclear cell effector functions, in particular, cell mediated cytotoxicity. We therefore explored general peripheral blood cell mediated cytotoxic capabilities in short term assays (up to 48 hours) and have found that both lectin induced cellular cytotoxicity (LICC) and spontaneous cell mediated cytotoxicity (SCMC) against certain targets are defective in naturally infected patients with malaria. If cytotoxic cells function in clearing the malaria parasite, then interference with normal cytotoxic effector mechanisms could be a way in which malaria parasites avoid destruction by the host immune system.

Previous studies by Greenwood and coworkers (1977) and by Brown and Smalley (1980) have reported increased nonspecific and specific antibody dependent cellular cytotoxicity by peripheral blood cells from Gambian children with malaria, which was felt to correlate with an increase in the number of Null cells also present in these patients (Wyler, 1976). Our finding of no difference in ADCC effector function between patients and controls may be explained in part by different antibodies, different target cell types, or by the different type of patient examined, in that we have studied naturally infected adults from a different geographical area than the previous studies. Furthermore, we have found in our same patients (Wells et al., 1979) that there is a decrease in T cells both in percentage and absolute number, and that although an increase in Null cell percentage was observed, there was not only no increase but actually a slight decrease in the absolute number of Null cells present, a finding which would correlate with not observing a change in ADCC. Therefore, adults from Thailand may express different, although internally consistent, immunologic changes in comparison to children from Gambia after natural malaria infection.

Present evidence indicates that ADCC, SCMC, and LICC effector cells belong to subsets of T as well as Null lymphocytes. SCMC and ADCC effector cells include non-adherent, non-phagocytic, surface membrane immunoglobulin-negative, Fc receptor positive, E rosette positive lymphoid cells (Hersey et al., 1975; West et al., 1977; Bolhuis et al., 1978; MacDermott et al., 1980b); SCMC but not ADCC can be mediated by an E-rosetting subset which lacks detectable receptors for Fc (Bolhuis et al., 1978); and both SCMC can be mediated by a subset of Null cells (MacDermott et al., 1980b). T cells with Fc receptors

for IgG; i.e., T_G cells (Moretta et al., 1976) are known to be capable of mediating SCMC against K562 cells (Gupta et al., 1978), while adherent monocytes and B lymphocytes appear not to be directly involved in SCMC cell lysis (Bolhuis et al., 1978; West et al., 1977; MacDermott et al., 1980b). Depending on the lectin used and the target cell type, LICC effector cells also belong to the Fc receptor positive populations (Bonavida et al., 1977, Bonnard and West, 1977), the T cell subclass (Nelson et al., 1976), Fc receptor-negative populations in the presence of PHA (Waller et al., 1976) and T, B, and Null cell subclasses (MacDermott et al., 1980b). However, LICC is also critically dependent upon the lectin used and the species from which the target cells are obtained (MacDermott et al., 1976). Furthermore, the subpopulation of human lymphocytes that responds to WGA may be quantitatively minor. Indeed, Boldt (1980) demonstrated that approximately 50% of the WGA bound to the surfaces of human lymphocytes can be localized to only 10% of the cells. This finding is also consistent with the data of Gordon et al., (1980), in that early blast transformation and DNA synthetic responses caused by WGA seems to involve only 10-15% of the T cells present.

There are several possible mechanisms to account for the decrease in lectin induced cellular cytotoxicity and spontaneous cell mediated cytotoxicity with normal antibody dependent cytotoxicity as observed in the present study. Firstly, as discussed above, different lymphocyte subclasses mediate each of the different types of cell mediated cytotoxicity. Thus a physical deletion or regulatory suppression of the appropriate subclasses could account for the results. For example, specific lymphocytotoxic antibodies could block or lead to deletion of the relevant T cells and a subgroup of NK cells but not K cells, if the Fc receptor is not blocked. Alternatively, antigens released from the malaria parasite organisms themselves could block the effector lymphocyte receptors involved in target cell recognition or lead to deletion of the lymphocyte subclass by antigen antibody complex formation on the surface of mononuclear cells followed by removal in the reticuloendothelial system. In addition it should be noted that the changes in immune function observed in the present study may be transient and reversible, thus indicating alterations of cell trafficking rather than cell deletion.

Because serum factors or immunoglobulins might account for the present data, it is of importance that no increase was seen in either SCMC or LICC activity when mononuclear cells from malarious patients were incubated overnight. These results suggest that membrane binding serum factors, such as lymphocytotoxic antibodies or immune complexes, which have been reported to exist in sera from some malaria patients (Wells et al., 1980; Adam et al., 1981), were not inhibiting SCMC or LICC. Furthermore, the finding that sera from patients with malaria does not inhibit SCMC or LICC effector cell functions by MNC from healthy individuals supports the conclusion that sera from malarious individuals has little or no *in vitro* effect on non-specific effector functions during the early stages of malaria infection. It still remains unclear, however, as to whether the impaired cytotoxic function is due to defective early differentiation of the respective cell subpopulations or a direct effect of parasite induced regulatory factors on the specific functional cell subpopulations.

The presence of immune complexes (Jewell and MacLennan, 1973) and other substances (Larsson et al., 1973) in sera that bind Fc receptors and inhibit ADCC *in vitro*, as well as the demonstration of immune complexes in malarious monkey plasma (Houba et al., 1976) suggested a possible serum regulator mechanism capable of altering ADCC activity *in vivo*. Therefore, in the present study we have also investigated the effect of patients' sera on ADCC by peripheral cells from healthy donors. Using the ADCC inhibition technique we failed to detect immune complexes or any inhibitory factor in sera from patients with *P. falciparum* and *P. vivax*. Since the ADCC inhibition technique is a sensitive method for detecting small size immune complexes (Meyer and Descamps, 1979) in serum, our results may indicate that either soluble immune complexes are rarely present in malarious sera or that the IgG subclasses responsible for the ADCC blocking phenomenon differ from those found in malarious sera immune complexes.

The finding of impaired cytotoxicity by the malarious patients adherent cells in the LICC assays, but not in the ADCC assays, may indicate several things. Firstly, the impaired LICC activity of the patients' unfractionated MNC was not due to inhibition by the macrophages (the adherent cell population routinely contained > 98% esterase positive cells), since LICC by the patients' macrophage depleted cells was also significantly impaired. Secondly, the apparent dysfunction of patients' macrophages in the LICC assays, may indicate that malaria infection can also result in the inhibition of the functional capabilities of macrophages to destroy some erythrocyte populations. This is of importance because a mechanism of immunity in malaria has been thought to be the opsonization of parasitized erythrocytes by macrophages.

Reduced LICC activity by macrophages from malarious humans is consistent, in part, with the report by Frankenburg et al., (1980) of decreased phagocytosis throughout the malaria infection by cells from lethally infected mice (non-immune). Furthermore, impaired macrophage LICC in malarious individuals may be associated with the decrease in T cell numbers known to exist in malarious individuals in that animal studies have demonstrated that T cells mediate the activation of macrophages (Mackness, 1960; Krahenbuhl et al., 1973). Hypothetically other subsets of T cells may be needed for the activation of additional macrophage functions and any decrease in the respective subpopulations of T cells would lead to an impairment of specific and non-specific macrophage functions.

It is of interest that the defects observed in the present study have also been described in diseases with a possible autoimmune basis such as multiple sclerosis (Benczur et al., 1980) and systemic lupus erythematosus (Silverman and Cathcart, 1980). The defects in cell mediated cytotoxicity in SLE and MS in conjunction with the numerous other similarities which we have noted between the immunologic changes in patients with malaria and those well described for patients with autoimmune diseases, may imply that part of the host response to a successful ongoing infectious or agent induced disease includes a decrease in cell mediated cytotoxicity, depressed T cell numbers, depressed suppressor T Cell function, suppression by serum factors, as well as the presence of lymphocytotoxic antibodies. The implication of this observation in our understanding of autoimmune diseases is that changes such as those observed in the present

study can occur secondary to a parasitic agent as well as in autoimmune processes such as systemic lupus erythematosus. On the other hand, the implication for the study of malaria is that the changes in immune function induced by parasitic infestation may allow or contribute to the autoimmune phenomena sometimes seen in these illnesses. Further studies on the mechanisms by which the malaria infection alters human immune function are of importance so that we can learn how parasites avoid or protect themselves from destruction or elimination by the host.

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Legends to Figures :

- Figure 1 Spontaneous cell mediated cytotoxicity by unfractionated mononuclear cells from malarious Thai adults against K562 target cells. Values expressed as mean percentage cytotoxicity \pm S.E.M. for the number of patients and concurrent controls studied (N). * indicates level of statistically significant difference between patient and control cell cytotoxicity.
- Figure 2 Spontaneous cell mediated cytotoxicity (SCMC) against Chang cells expressed as mean percent cytotoxicity \pm S.E.M. for the number of patients and concurrent controls (N) studied. Effector cells are from *P. vivax* patients (a) and *P. falciparum* patients (b). Open bars show the control data and cross hatched bars show the patient data.
- Figure 3 Antibody dependent cellular cytotoxicity against Chang liver target cells expressed as mean percent cytotoxicity \pm S.E.M. for the number of patients and concurrent controls studied (N). Cytotoxicity observed with cells alone (SCMC) was subtracted from that seen with cells plus rabbit anti-Chang antibody. Effector cells were from *P. vivax* patients (a) and *P. falciparum* patients (b).
- Figure 4 Antibody dependent cellular cytotoxicity against chicken red blood cells expressed as mean percent cytotoxicity \pm S.E.M. for the number of patients and concurrent controls studied (N). Effector cells were from *P. vivax* patients (a) and *P. falciparum* patients (b). Cytotoxicity observed with cells alone (SCMC) was subtracted from that seen with cells plus rabbit anti-CRBC antibody. Antibody alone was not cytotoxic.
- Figure 5 Lectin induced cellular cytotoxicity by unfractionated mononuclear cells from malarious Thai adults against human red blood cell targets. Values expressed as mean percentage cytotoxicity \pm S.E.M. for the number of patients and concurrent controls studied (N). * indicates level of statistically significant difference in patient and control cell cytotoxicity. N.S. indicates no statistically significant difference.
- Figure 6 Relationship of individual patient's MNC's SCMS (K562) and LICC (HRBC-WGA) response in respect to SCMC and LICC responses of MNC from their respective age-matched control. The range mean \pm 10% of the cytotoxic activity of the corresponding normal control is arbitrarily considered as normal. All assays were performed using a 25:1 effector to target cell ratio.
- Figure 7 Lectin induced cellular cytotoxicity (a) and spontaneous cell mediated cellular cytotoxicity (b) of unfractionated, non-adherent or adherent mononuclear cell populations from malarious Thai adults. Effector cells were from both *P. falciparum* and *P. vivax* patients.

Cytotoxicity is expressed as mean percent cytotoxicity \pm S.E.M. for the number of patients and concurrent controls studied (N). * indicates level of statistically significant difference in cytotoxicity between the patient and control cell populations at the respective effector to target cell ratio.

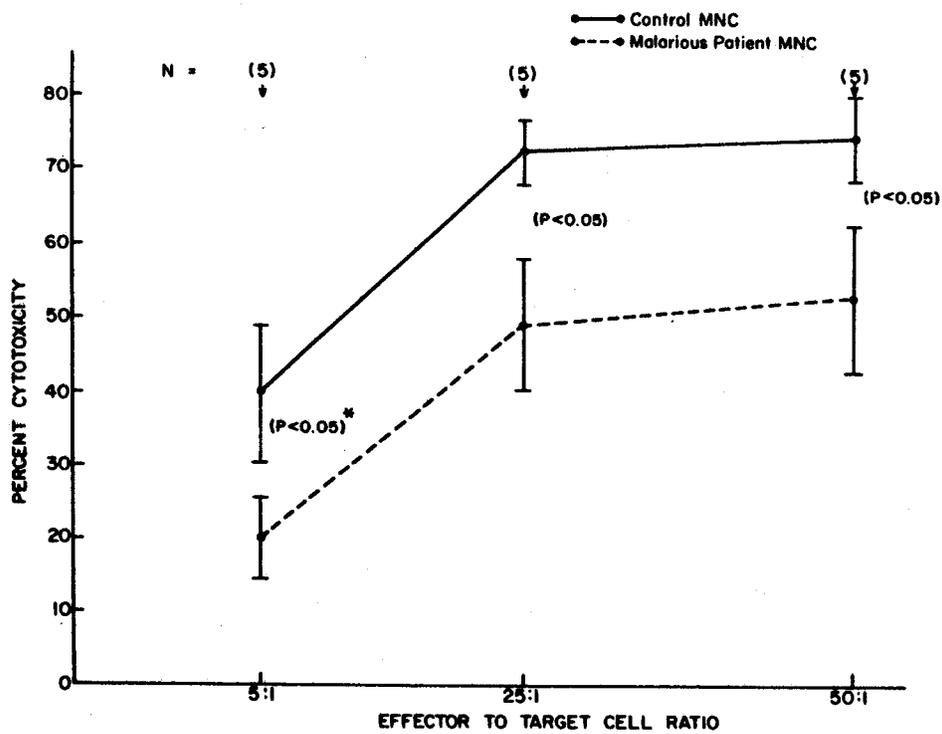


Figure 1. Spontaneous cell mediated cytotoxicity by unfractionated mononuclear cells from malarious Thai adults against K562 target cells.

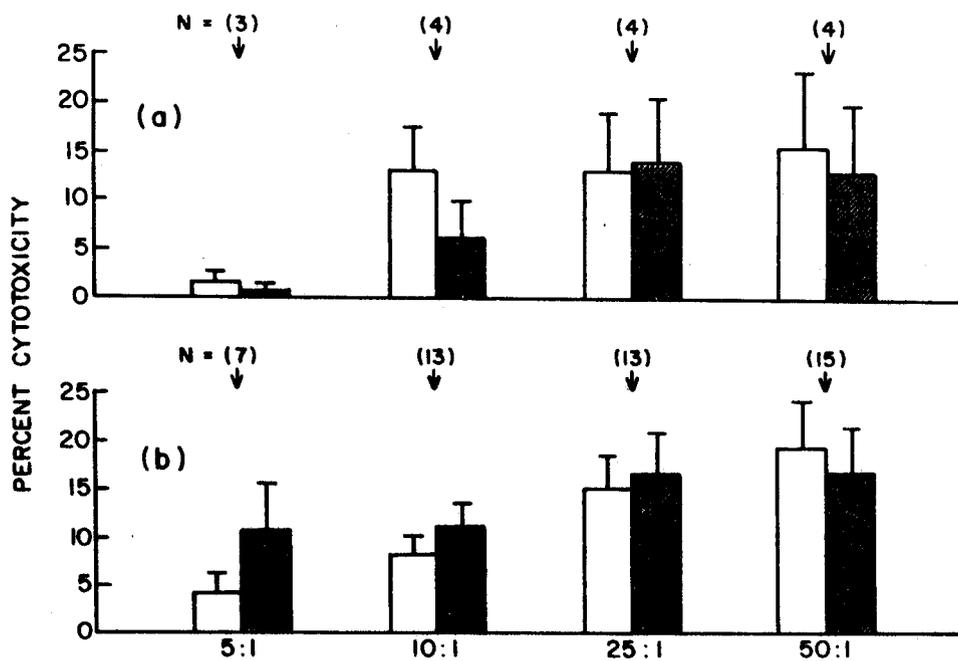


Figure 2. Spontaneous cell mediated cytotoxicity (SCMC) against Chang cells expressed as mean percent cytotoxicity \pm S.E.M. for the number of patients and concurrent controls (N) studied.

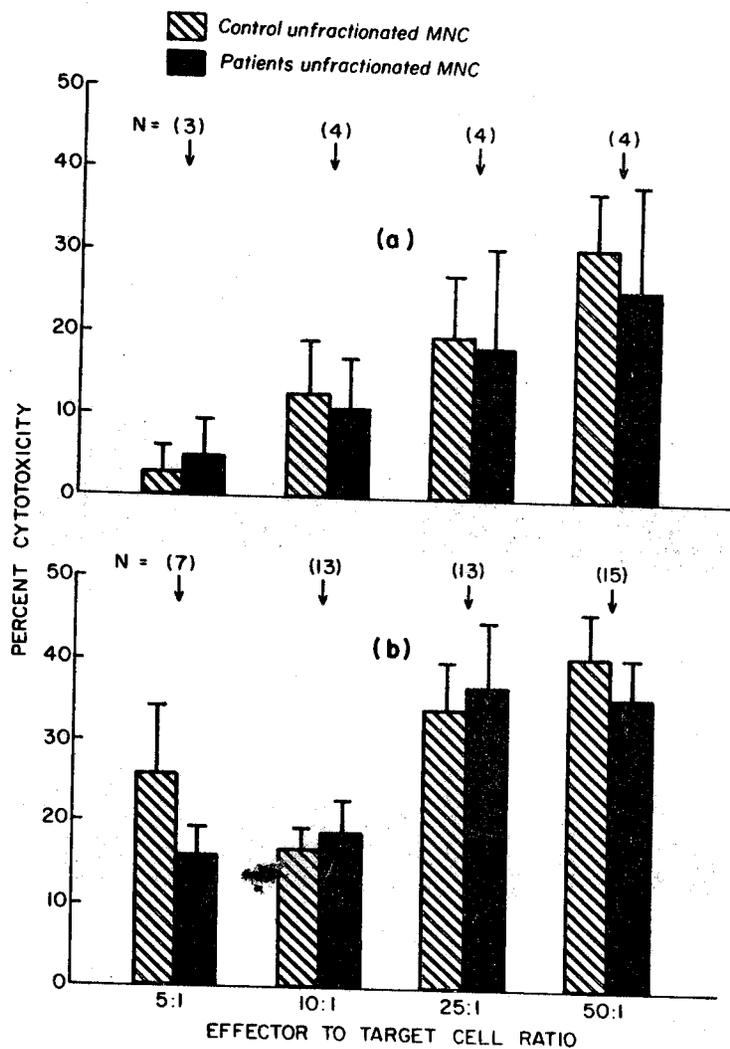


Figure 3. Antibody dependent cellular cytotoxicity against Chang liver target cells expressed as mean percent cytotoxicity \pm S.E.M. for the number of patients and concurrent controls studied (N).

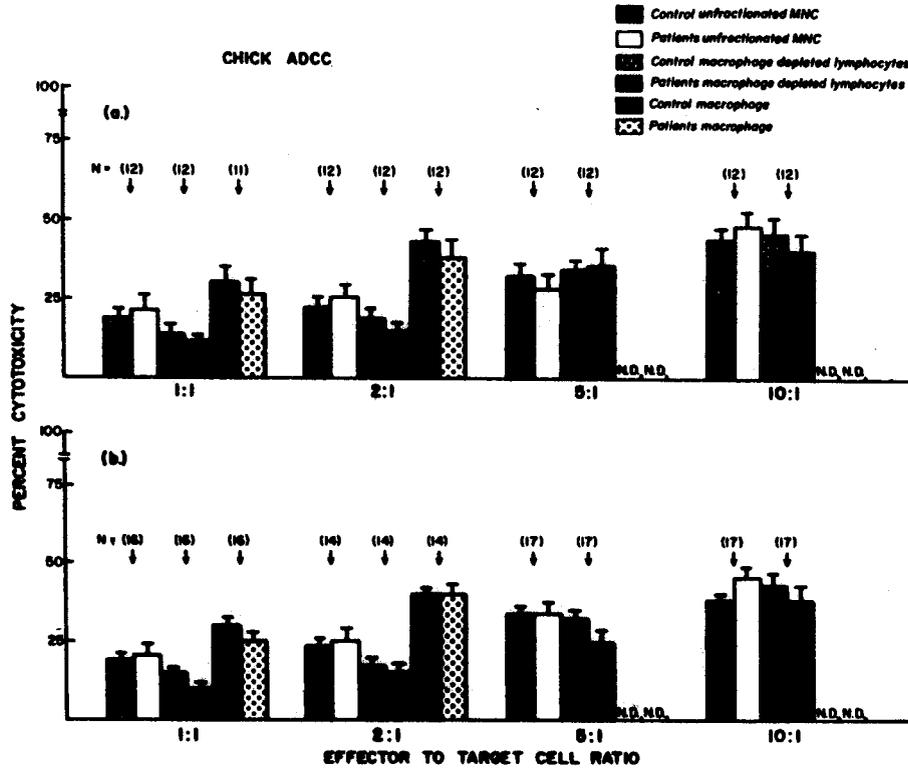


Figure 4. Antibody dependent cellular cytotoxicity against chicken red blood cells expressed as mean percent cytotoxicity \pm S.E.M. for the number of patients and concurrent controls studied (N).

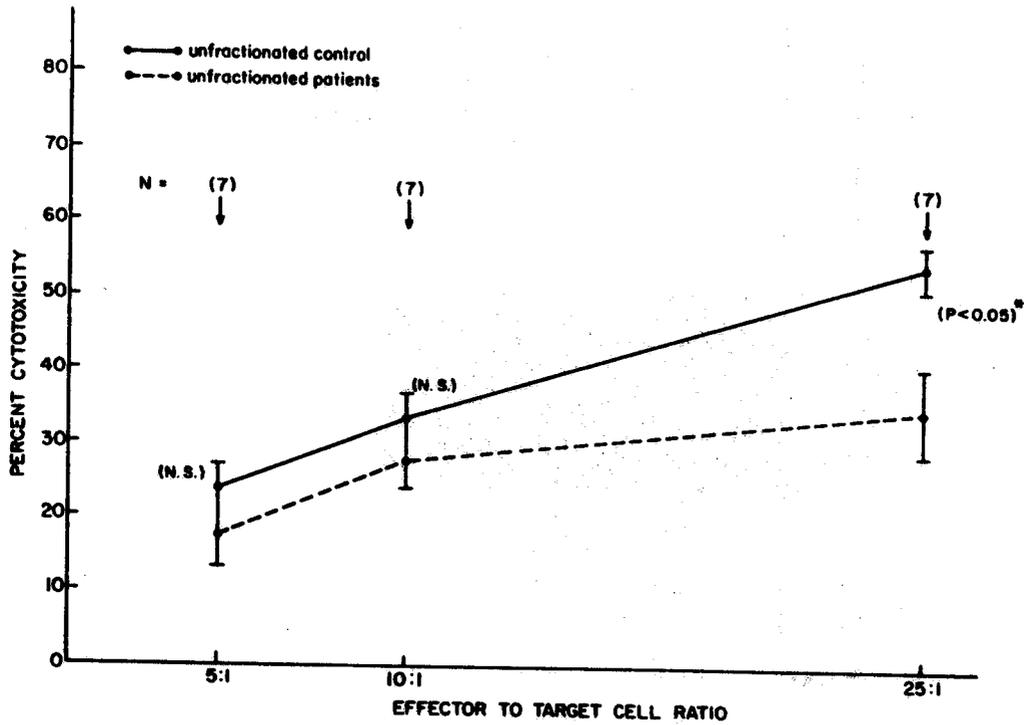


Figure 5. Lectin induced cellular cytotoxicity by unfractionated mononuclear cells from malarious Thai adults against human red blood cell targets.

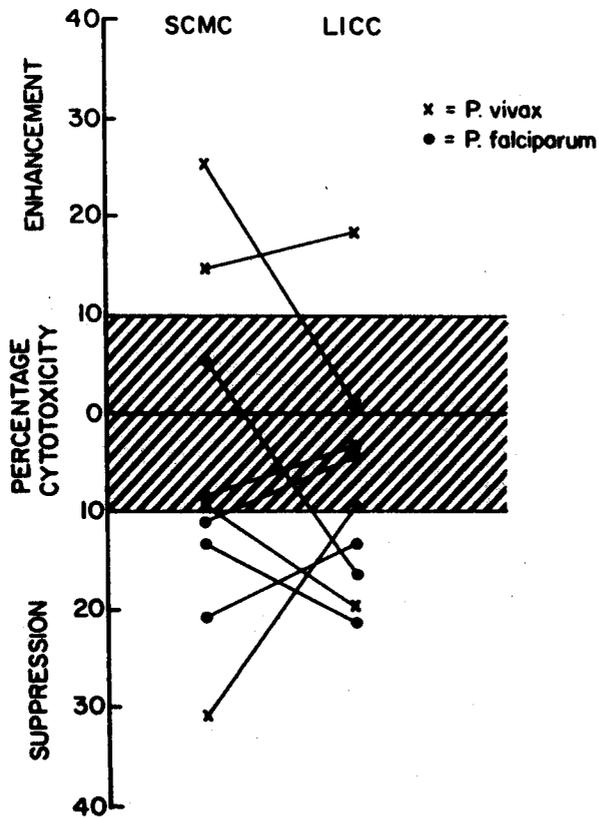


Figure 6. Relationship of individual patient's MNC's SCMC (K562) and LICC (HRBC-WGA) responses in respect to SCMC and LICC responses of MNC from their respective age-matched control.

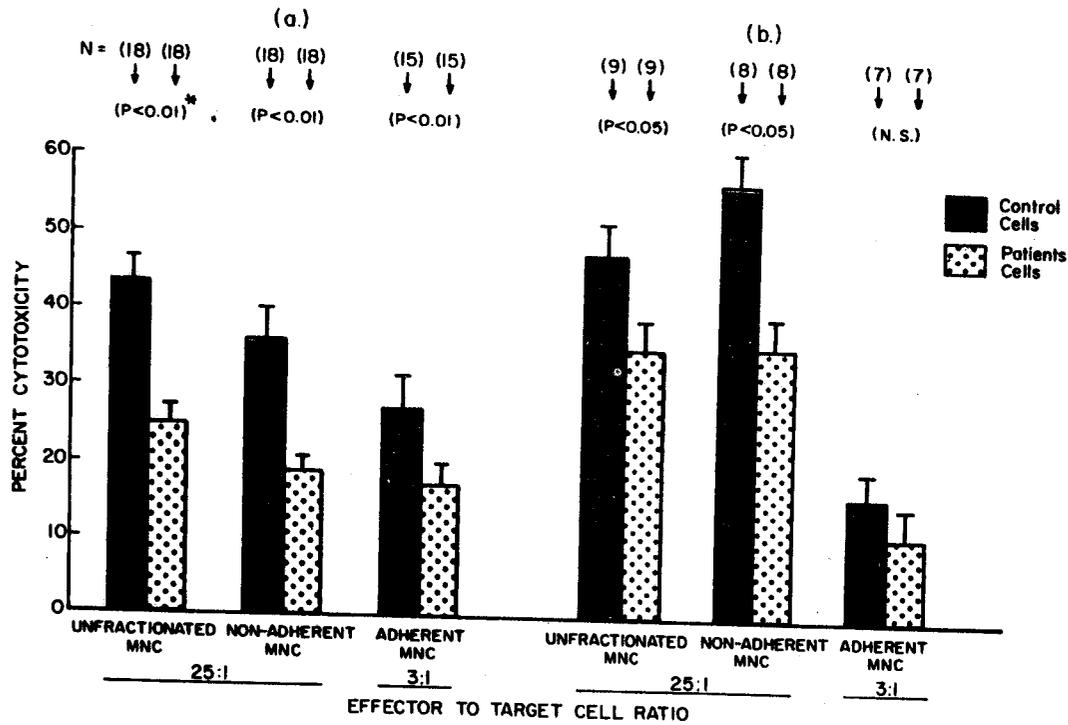


Figure 7. Lectin induced cellular cytotoxicity (a) and spontaneous cell mediated cellular cytotoxicity (b) of unfractionated, non-adherent or adherent mononuclear cell populations from malarious Thai adults.

Table 1. Spontaneous cell mediated cytotoxicity (SCMC), Lectin induced cellular cytotoxicity (LICC), and antibody-dependent cellular cytotoxicity (ADCC) by unfractionated MNC from Thai adults with naturally acquired *P. falciparum* malaria infection using Chang cell line cells as targets.

		<u>Control</u>	<u>Patients</u>
50:1 ^b	Spontaneous (SCMC)	33.2 ± 8.0 ^a	29.1 ± 9.6
	PHA ^c (LICC)	55.1 ± 11.2	29.9 ± 5.8 ⁺⁺
	Anti-Chang Ab ^d (ADCC)	58.4 ± 5.8	51.5 ± 9.1
25:1	Spontaneous (SCMC)	23.9 ± 5.4	23.8 ± 9.1
	PHA (LICC)	45.7 ± 8.3	27.5 ± 6.8 ⁺
	Anti-Chang (ADCC)	51.2 ± 7.4	52.7 ± 13.6
10:1	Spontaneous (SCMC)	14.1 ± 3.8	19.0 ± 5.0
	PHA (LICC)	29.2 ± 2.9	23.2 ± 6.4
	Anti-Chang Ab (ADCC)	23.2 ± 4.4	27.8 ± 6.3

^a Mean ± S.E.M. percent ⁵¹Cr release for six individuals.

^b Effector to target cell ratio.

^c Final concentration of PHA (1 µg/ml).

^d Final dilution of 1:3000 rabbit anti-Chang serum.

⁺ Statistically significant difference (p < 0.05).

⁺⁺ Statistically significant difference (p < 0.01).

Table 2. ADCC of normal peripheral blood MNC against CRBC or Chang target cells after treatment with malarious patient serum.

<u>Targets</u>	<u>Control Serum</u>	<u>Patient Serum</u>	
<u>Chick RBC</u>			
1:1 ^a	11.9 ± 5.1 (14) ^b	9.2 ± 4.9 (14)	N.S. ^c
2:1	14.1 ± 5.2 (14)	7.7 ± 1.8 (14)	N.S.
10:1	29.4 ± 7.3 (14)	20.9 ± 4.3 (14)	N.S.
<u>Chang</u>			
5:1	14.4 ± 3.3 (9)	22.0 ± 5.0 (9)	N.S.
10:1	23.9 ± 1.8 (5)	34.2 ± 5.6 (5)	N.S.
25:1	17.6 ± 9.5 (3)	39.5 ± 16.2 (3)	N.S.

^a Effector to target cell ratio.

^b Mean percent cytotoxicity ± S.E.M. for the number of individual experiments (in parenthesis).

^c Difference in mean percent cytotoxicity not statistically significant ($p \geq 0.05$ by the Students t test)

Table 3. Effect of malarious patients sera on normal and patient MNC in SCMC and LICC assays

Experiment Number	SCMC			LICC		
	1	2	3	4	5	6
<u>Control MNC</u>						
A. Fresh cells ^a	54 ± 3 ^{ob}	47 ± 2 ^o	63 ± 4 ^o	42 ± 3*	59 ± 3*	46 ± 4*
B. After overnight incubation	22 ± 2	60 ± 5	60 ± 3	43 ± 3	44 ± 2	18 ± 3
C. After overnight incubation ^c and subsequent addition of						
(1) autologous serum	23 ± 2	56 ± 3	54 ± 2	43 ± 3	44 ± 4	24 ± 3
(2) patient serum	26 ± 5	41 ± 4	47 ± 4	31 ± 3	40 ± 5	17 ± 4
<u>Patient MNC</u>						
A. Fresh cells ^a	51 ± 3	27 ± 3	63 ± 4	24 ± 3	36 ± 2	61 ± 3
B. After overnight incubation	30 ± 2	10 ± 3	49 ± 2	27 ± c	16 ± 3	5 ± 2
C. After overnight incubation ^c and subsequent addition of						
(1) autologous serum	26 ± 2	11 ± 3	46 ± 3	7 ± 2	14 ± 2	2 ± 0.7
(2) age-matched control serum	23 ± 3	17 ± 2	43 ± 4	15 ± 2	16 ± 2	3 ± 1

^o Designates that *P. falciparum* patients cells and serum or

* *P. vivax* patients cells and serum were used in the experiment.

^a SCMC and LICC of fresh cells was determined as described under Materials and Methods

^b Numbers represent the mean ± S.E.M. of triplicate determinations and are corrected for mean spontaneous release.

^c Cells were incubated as described under Materials and Methods and washed once before SCMC and LICC was determined.

Table 4. Effect of malarious patient serum on SCMC and LICC by nonadherent mononuclear cells from healthy donors

	% Cytotoxicity		LCA > 40%
	SCMC	LICC	
MNC + Media	43.3 ± 0.3	30.3 ± 0.9	-
+ P.f. serum #1	42.0 ± 3.3	N.D.	+
+ P.f. serum #2	39.6 ± 1.6	N.D.	-
+ P.v. serum #1	38.5 ± 0.6	N.D.	+
+ P.v. serum #2	41.6 ± 0.4	N.D.	-
+ P.f. serum #3	N.D.	32.4 ± 0.6	+
+ P.f. serum #4	N.D.	25.3 ± 1.0	-
+ P.v. serum #3	N.D.	25.8 ± 0.9	+
+ P.v. serum #4	N.D.	23.2 ± 2.7	-
+ Pooled control sera	40.7 ± 1.3	28.9 ± 3.7	-
+ FCS	40.0 ± 1.8	26.5 ± 0.4	-
+ Autologous serum	40.2 ± 2.9	23.2 ± 0.4	-

All SCMC or LICC assays were performed at the same time. Numbers represent the mean ± S.E.M. of triplicate determinations and are corrected for mean spontaneous release.

Effector MNC were incubated with an equal volume of heat-inactivated serum and the assay performed as described under Materials and Methods.