

MEASUREMENT OF ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY
(ADCC) BY PERIPHERAL BLOOD MONONUCLEAR CELLS
FROM PATIENTS WITH NATURALLY ACQUIRED
P. falciparum OR *P. vivax* MALARIA

Principal Investigators : Barnyen Permpanich
Micheal J. Gilbreath, CPT, MSC
Katchrinnee Pavanand, M.D.
Prasit Sookto

Associate Investigators : Somchit Tulyayon
Somchai Kongchareon
Niphon Chuannak

OBJECTIVE : To investigate the antibody dependent cellular cytotoxicity capabilities of mononuclear cells from patients infected with malaria.

BACKGROUND : Antibody dependent cellular cytotoxicity (ADCC) is a phenomenon measurable *in vitro* which involves the destruction of IgG coated target cells by a recently defined population of non-specific effector cells known as K cells (1). The term K cell is more of an operational definition than a designation for a particular population of lymphocytes, and the K cells account for less than 5% of the total lymphocyte population in human peripheral blood.

It has been reported that cells responsible for ADCC activity increase in both humans and mice during malarial infection (2, 3). However, Wells, et al. (4) recently showed that although T cell subpopulations in humans infected with malaria were reduced, they found no alteration in the K cell (Null) or B cell subpopulations.

In our search for a correlation between assays of cellular immunity and protection against, or immune regulation by malaria parasites in humans, we looked at ADCC in Thais with naturally acquired malaria.

We investigated this response using the Chang target cell system and the Chick Red Blood Cell (CRBC) system. In addition we have examined the effect that coating of patient and/or control effector cells with sera obtained from malarious individuals has on ADCC.

METHODS : Peripheral blood was obtained from normal volunteers or malarious patients who were mildly ill, and recently infected, with *P. falciparum* or *P. vivax*.

Mononuclear cell populations were obtained from heparinized blood, Ficoll-Hypaque as previously described (5), and further fractionated using G-10 Sephadex bead into macrophage depleted or macrophage enriched effector cells for utilization in the CRBC target cell system. Unfractionated effector cells were used in Chang target cell system.

⁷ Rabbit anti-Chang serum was prepared by multiple intravenous injections of ¹⁰ Chang liver cells. Serum was collected 1-3 months after the first injection and heat inactivated at 56°C for 30 min.

Rabbit anti CRBC was obtained from Cappel Laboratories (Cochranville, Pa.).

In the cytotoxicity test procedure target cell damage was assessed by release of ⁵¹Cr from labelled target cells. Quantitative comparisons were made only within the experiments. Conditions within the experiment were kept rigidly constant; however, conditions varied to some extent from experiment to experiment.

In some assays serum from malarious patients were added and the effect on ADCC was compared with ADCC in identical cultures that received serum obtained from healthy volunteers.

Cytotoxicity was defined following the practice of Holm and Perlmann (6).

RESULTS : Little difference is seen between the ADCC activity between macrophage depleted cell fractions obtained from healthy volunteers and malarious patients with naturally acquired *P. falciparum* (Figure 1) or *P. vivax* malaria (Figure 2).

Similar results are seen when patient or control mononuclear cells are assayed for ADCC activity in the Chang target cell system (Figure 3).

Although small differences exist between ADCC activity of patient and control cells in some experiments, additional data are needed to determine if the difference is statistically significant.

To date 14 malaria sera samples have been tested in the Chick RBC system to determine if malaria sera can alter ADCC activity of macrophage depleted mononuclear effector cells from healthy volunteers. No significant difference has been found at any of the effector/target cell ratios ranging from 1:1 to 50:1. Work is continuing in this area.

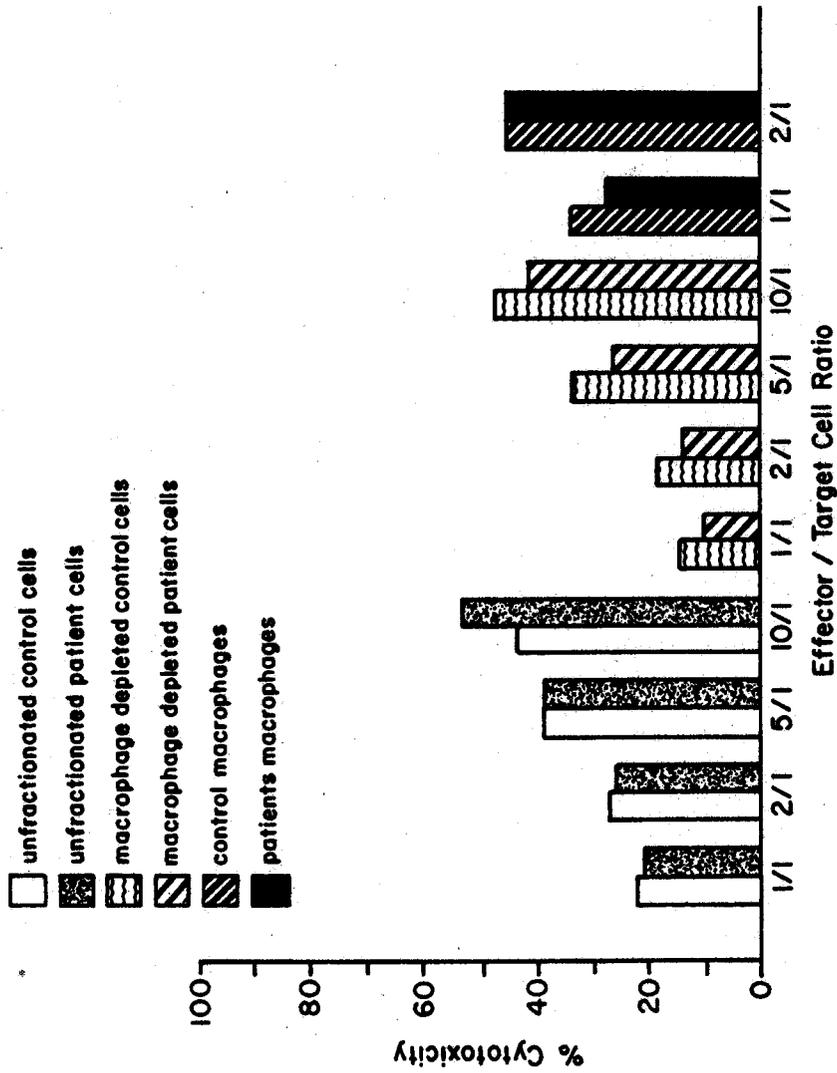


Figure 1. Average ADCC of Chick RBC Target Cells when peripheral bloods cells from Individuals with Naturally Acquired *P. falciparum* malaria or control volunteers were used as effector cells. (18 cases)

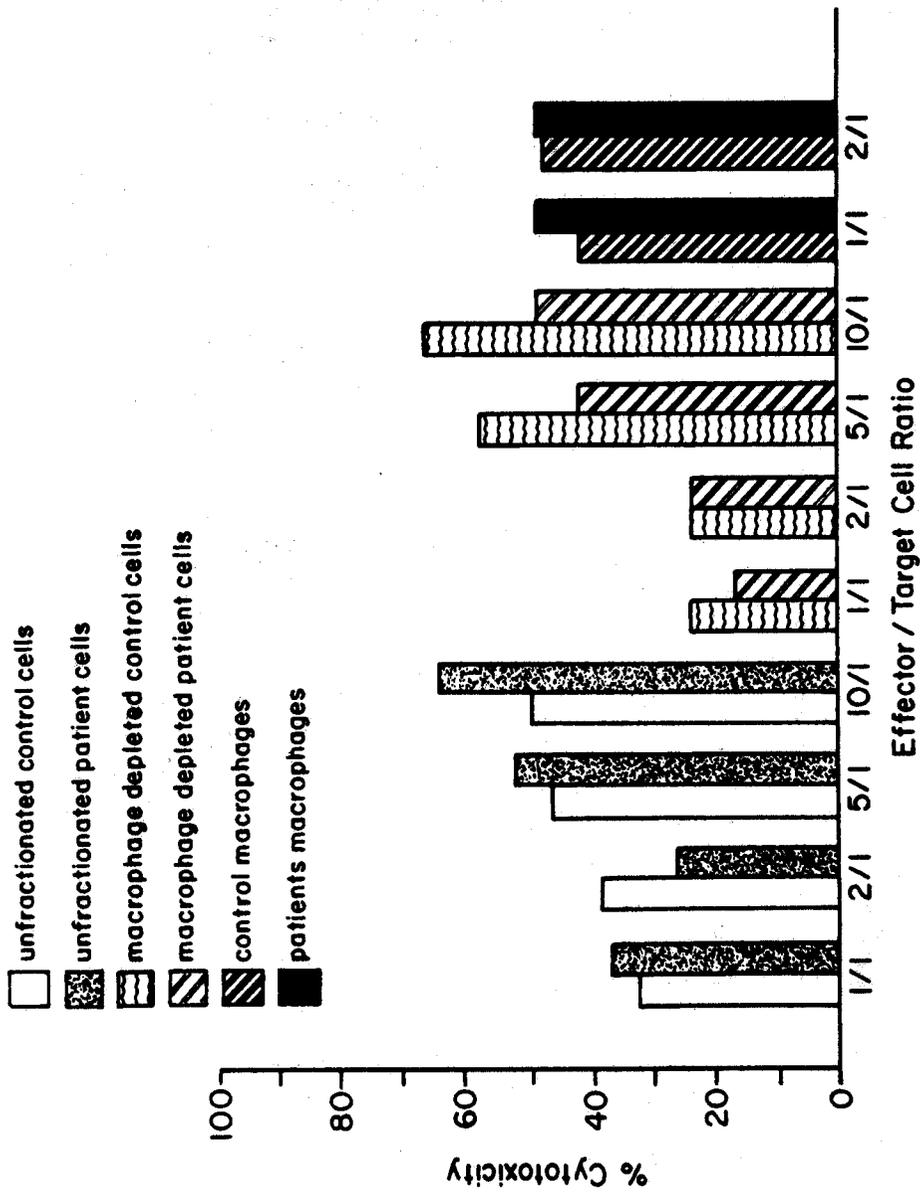


Figure 2. Average ADCC of Chick RBC Target Cells when peripheral blood cells from individuals with naturally acquired *P. vivax* malaria or control volunteers were used as effector cells (4 cases)

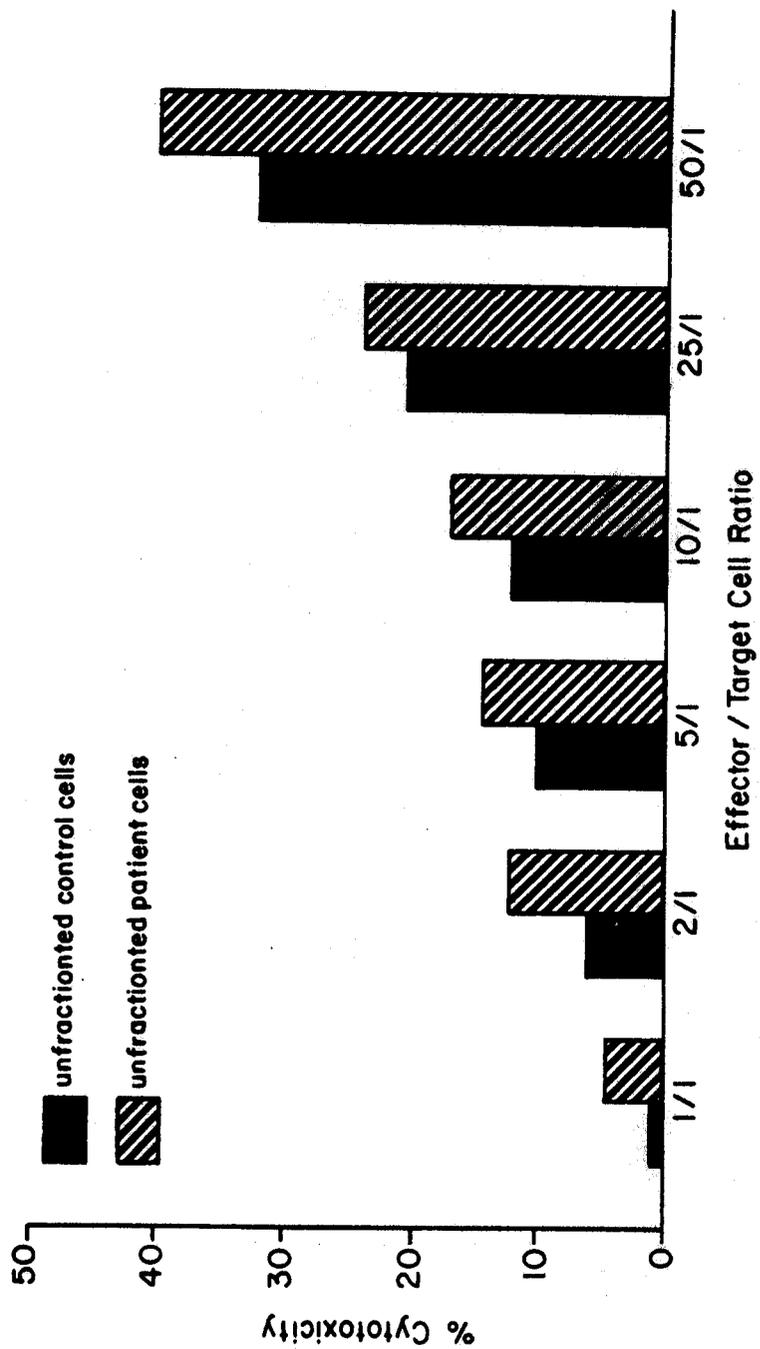


Figure 3. Average ADCC of 5 malarious patients (4 *P. falciparum*, 1 *P. vivax*) peripheral blood mononuclear cells when assayed using changing liver target cells.

REFERENCES :

1. Perlmann, P. H. Perlmann, A. Larsson and B. Wahlin : Antibody Dependent Cytolytic Effector Lymphocytes (K Cells) in Human Blood. *J. Reticulo. Soc.* 17: 241-250, 1975.
2. Greenwood, B.M., A.J. Oduloju and D. Stratton : Lymphocyte Changes in Acute Malaria. *Trans. Roy. Soc. Trop. Med. Hyg.* 71: 408, 1977.
3. MacDonald, V. and R. Phillips : Increase to Non-specific Antibody Mediated Cytotoxicity in Malarious Mice. *Clin. Exp. Immunol.* 34: 159-163, 1978.
4. Wells, R.A., K. Pavanand, S. Zolyomi, B. Permpnich and R.P. MacDermott : Loss of Circulating T Lymphocytes with Normal Levels of B and "Null" Lymphocytes in Thai Adults with Malaria. *Clin. Expl. Immunol.* 35: 202, 1979.
5. Gilbreath, M.J. et al. : Nature of Malaria Cold-Reactive Lymphocytotoxic Antibody. *AFRIMS Annual Progress Report*, 1980.
6. Holm, G. and P. Perlmann : Quantitative Studies on Phytohemagglutinin Induced Cytotoxicity by Normal Human Lymphocytes Against Homologous Cells in Tissue Culture. *Immunology* 12: 525, 1967.