

Transformation of Patient Lymphocytes by Selected Malaria Antigens

Principal Investigators : Robert A. Wells, MAJ, MSC
Sandor Zolyomi, SFC, USA
Pirom Phisphumvithi

Associate Investigators : Somchai Kokchareon
Theera Wimonwattrawatee

OBJECTIVE : To develop preliminary methodology for the direct stimulation of malaria patient lymphocytes by erythrocyte and sporocyte antigens as a basis for more definitive studies.

BACKGROUND : The availability of assays with sufficient specificity and sensitivity presents a major barrier to the understanding of host immune response mechanisms in malaria. There are several recent reports in the literature which may assist in the solution of this problem. Of special interest are papers dealing with the *in vitro* stimulation of sensitized lymphocytes by malaria antigen employing either rodent (1, 2) or human material (3). This report summarizes the results of pilot studies employing similar techniques with material from Thai malaria patients. These assays are critical to the success of more definitive heterogeneity studies using cells and antigens from different geographic regions.

METHODS : Isolated mononuclear leukocytes were cultured in modified RPMI 1640 media according to the methodology of MacDermott et al. (4). Assays for lymphocytes from *falciparum* (P.F.) infections used lymphocytes from P.F. patients and P.F. antigen extracted from infected chimpanzee erythrocytes. Assays for *vivax* infections (P.V.) were done with lymphocytes from *vivax* infections and P.V. sporozoites. Controls consisted of cells and media not containing antigen. Cell suspensions were incubated with antigen for 6 days at 37°C in 5% CO₂. Cultures were then pulsed with 0.4 uCi ³H thymidine and further incubated for 24 hours. The cells were then isolated and washed by a multiple automated sample harvester (MASH). After drying, individual filter pads containing the cells were placed in scintillation vials containing hydromix and were counted (CPM) in a Hewlett-Packard beta scintillation counter.

RESULTS : The findings of 8 preliminary assays are summarized in Tables 1 and 2. The stimulation ratios (SR) of patient lymphocytes vs controls were calculated by the following formula :

$$SR = \frac{\text{CPM test}}{\text{CPM control.}}$$

Table 1 illustrates the values of 5 assays conducted in P.F. system. The SR range was 1.5 - 3.7 (mean 2.4). The SR values of 3 assays thus far conducted with the P.V. system are indicated in Table 2. Here the range was 1.4 - 2.3

(mean 2.0). With both approaches it is clear that further work is necessary to develop the assay. Modifications are underway which involve both cell and radioisotopes concentrations are incubation time. This project continues.

REFERENCES :

1. Weinbaum, F.I., Evans, C.B. and Tigelaar, R.E. : An In Vitro Assay for T Cell Immunity to Malaria in Mice. *J. Immunol.*, 116:1280-1283, 1976.
2. Golenser, J. et al. : Specific Lymphocyte Transformation in Murine Malaria. *Z. Parasitenk.*, 50:95-98, 1976.
3. Wyler, D.J. and Oppenheim, J.J. : Lymphocyte Transformation in Human *Plasmodium falciparum* Malaria. *J. Immunol.*, 113:449-454, 1974.
4. MacDermott, R.P., Chess, L. and Schlossman, S.F. : Immunologic Functions of Isolated Human Lymphocyte Subpopulations. V. Isolation and Functional Analysis of a Surface Ig Negative, E Rosette Negative Subset. *Clin. Immunol. and Immunopath.*, 4:415-424, 1975.

Table 1. Stimulation Ratios : *P. falciparum* Antigen
(Chimpanzee/RBC) x human *falciparum* Lymphocytes

Assay #	Stimulation Ratio
1	2.2
2	2.3
3	3.7
4	1.5
5	2.2
Range	1.5 - 3.7
Mean	2.4

Table 2. Stimulation Ratios : *P. vivax* Antigen
(human/sporozoite) x human *vivax* Lymphocytes

Assay #	Stimulation Ratio
1	1.4
2	2.3
3	2.3
Range	1.4 - 2.3
Mean	2.0