T Lymphocyte Subpopulations in Thai Malaria Patients

Principal Investigators : Robert A. Wells, MAJ, MSC
Katchrinnee Pavanand, M.D.
Sandor Zolyomi, SFC, USA
Barnyen Permpanich

Associate Investigators : Somchai Tulyayon
Prasit Sookto
Niphon Chuanak

OBJECTIVE : To determine changes in the proportions of subpopulations of T lymphocytes of malaria patients in comparison with those of normal volunteers.

BACKGROUND : In a previous study conducted in this laboratory (1) it was determined that in human malaria patients there is depression in the percentage of circulating T lymphocytes as determined by the sheep red cell (E) technique. As an extension of this study we wished to determine whether these alterations involved only one subpopulations of lymphocytes or whether such changes were associated with all subpopulations of T cells. Current methodology allows for the isolation of lymphocyte subpopulations by physico - chemical technique (2, 3). It was thus decided to adopt these techniques to the study of subpopulations of malaria patient lymphocytes.

METHODS : In the course of these experiments the methodology of Glinski et al. (3) was employed with modification. Each sample of heparinized blood was centrifuged at 400 x g for 30 minutes in the cold. The resulting leukocyte rich layer was removed by Pasteur pipette and, after washing, the cells were standardized to a concentration of 2×10^6 mononuclear cells/ml in Hank's balanced salt solution (HBSS). A 0.1 ml sample was assayed for the percentage of T lymphocytes forming E rosettes with sheep erythrocytes. The remainder of each aliquot of cell suspension was processed by discontinuous ficoll-hypaque centrifugation. Samples were layered on a "column" of ficoll of graded concentration ranging from 30% ficoll at the bottom of the polycarbonate centrifuge tube to 9% at the top. Samples were centrifuged as before. Each centrifugation yielded seven cell fractions which were sequentially drawn off, washed and standardized in HBSS. Due to a scarcity of cells, the first 3 strata were pooled. Each aliquot was then standardized as before and studied for the percentage of E rosettes formed. All assays were made blind with the origin of the sample unknown to the technician. The percentages of E rosettes were calculated from duplicate hemocytometer readings and the results averaged. In addition to determining the mean values for patients and normal controls, the relative decrease of the two populations was determined by the following formula :

$$\text{Relative decrease} = 100\% - \left(\frac{\text{Patient mean}}{\text{Normal mean}} \times 100 \right) .$$

RESULTS : Table 1 summarizes the E rosette values for all T cell assays conducted. As in the previous studies cited (1) the T lymphocyte values of unfractionated samples were suppressed in malaria patients. The data indicated for each subfraction both a slight absolute suppression (mean values) and a relative decrease in the patient T cell subpopulations (mean values) in comparison with those from normal controls. There was also a suggestion that cells of lower density were more suppressed than those of greater density. A manuscript on this work has been submitted for publication. This project is complete.

REFERENCES :

1. Wells, R.A. et al. Human Peripheral Blood Lymphocytes in Adults from Thailand with Naturally Acquired *Plasmodium falciparum* and *Plasmodium vivax*. Manuscript in preparation.

2. Tak Yan Yu. et al. Lymphocyte Populations : Separation by Discontinuous Density Gradient Centrifugation. J. Immunol. 110:1615-1622, 1973.

3. Glinski, W., Gershwin, M.E., and Steinberg, A.D. Fractionation of Cells on a Discontinuous Ficoll Gradient : Study of Subpopulations of Human T Cells Using Anti-T-Cell Antibodies from Patients with Systemic Lupus Erythematosus. J. Clin. Invest. 57:604-614, 1976.

Table 1. Curculating T Lymphocyte Subpopulations in Malaria Patients

Assay No.	Percentage of E Rosettes After Ficoll-Hypaque Centrifugation											
	Unfractionated samples		Pooled subfractions A B C		Separate subfractions D		E		F		G	
	NC*	P+	NC	P	NC	P	NC	P	NC	P	NC	P
1	ND‡	ND	6	5	7	7	12	10	9	11	6	7
2	ND	53	7	4	8	6	11	8	11	9	7	5
3	ND	55	4	6	6	6	10	10	11	10	7	6
4	58	54	6	5	6	6	11	10	10	10	6	6
5	60	52	5	5	7	6	9	10	11	11	5	6
6	59	54	5	5	6	7	10	10	11	10	6	7
7	60	55	6	5	9	6	11	10	10	10	7	6
8	59	55	6	5	8	6	11	11	10	10	7	5
9	60	52	6	4	8	6	11	10	11	10	7	6
10	61	53	6	6	8	7	10	10	11	11	7	8
Mean	59.7	53.7	5.7	5.0	7.3	6.3	10.5	9.9	10.5	10.2	6.5	6.2
S.D.	±0.98	±1.23	±0.82	±0.67	±1.06	±0.48	±1.75	±0.74	±0.71	±0.63	±0.71	±0.92
Relative decrease (%)	10.1		12.3		13.7		5.7		2.9		4.6	

* NC = Normal control
\+ P = Patient

‡ ND = Not done
P. vivax patient, others are *P. falciparum*