

## Responsiveness of Malaria Patient Leukocytes in Mixed Leukocyte Culture (MLC)

Principal Investigators : Robert A. Wells, MAJ, MSC  
Katchrinnee Pavanand, M.D.  
Pirom Phisphumvidhi

Associate Investigators : Somchai Kokchareon  
Theera Wimonwattrawatee

**OBJECTIVE :** To evaluate general responsiveness of malaria patient leukocytes to stimulation with allogeneic white cells.

**BACKGROUND :** The responsiveness of one population of white cells to culture with those of an allogeneic source is well established. This methodology has been applied primarily in tissue typing research utilizing *in vitro* cellular recognition (stimulation) as a parameter for predicting *in vivo* organ transplant rejection. More recently, the characteristics of cells participating in this reaction (1, 2) and factors modulating the reaction (3) have been described.

**METHODS :** Mononuclear cells were isolated by ficoll hypaque centrifugation according to the methodology of Boyum (4). Stimulator cells were prepared by treating cells in RPMI 1640 media with 50 ug/ml mitomycin C at 37°C for 45 minutes. After washing ( $\times 3$ ) in SBSS the cell were adjusted to  $2.5 \times 10^6$ /ml in media. In the initial experiments one way cell crosses were performed between one set of patient cells and one set of normal cells. In a later series there was one set of patient cells with two sets of normal cells. In addition to replicates (6 each) for stimulation of the patient cells by normal cells and normal cells by patient cells, controls were provided for background nonspecific activity, efficacy of mitomycin C treatment and responsiveness to nonspecific stimulation (to PHA). All cell cultures were incubated at 37°C in 5% CO<sub>2</sub> for 5 days. The cells were then pulsed with (0.5 ug) <sup>3</sup>H-thymidine for 24 hours. All cells were placed on filter pads in hydromix. Counts were determined in a Hewlett-Packard beta scintillation counter and stimulation indexes were determined.

**RESULTS :** Table 1 summarizes the results of cell mixtures of single populations of patient cells with single populations of normal cells. There developed a consistent pattern in which patient cells showed elevated response values in comparison with those of the normal cells. It was uncertain as to whether this difference was due to a true difference or due to the inability of the normal cells to respond to other cell surfaces effectively. The experiments summarized in Table 2 were performed to answer this question. Here it is seen that both normal and patient cells respond comparably and that thus foreign surface antigens of any cell type can be recognized. Therefore, although the absolute numbers of circulating patient lymphocytes are reduced (as reported elsewhere), when standardized, these cells maintain the ability to recognize allogeneic markers. A manuscript on this work is in preparation. This project is complete pending redesign of experiments for more definitive investigations. This is a final report.

Table 1. - Mixed leukocyte culture in malaria  
 - One normal cell population with one patient cell population

Assay #	Normal Cell Responders	Patient Cell Responders
1	7.0*	2.7
2	2.2	4.9
3	1.1	3.6
4	1.1	14.5
5	1.1	34.3
6	2.3	9.1
7	1.9	31.5
8	13.6	15.6
9	6.2	9.0
10	8.6	12.8
11	12.2	22.2
12	8.0	5.1
13	2.4	2.8
14	2.5	15.4
Range :	1.1-13.6	2.7-34.3
Mean :	5.0	13.1

\* Stimulation index

Table 2. Mixed leukocyte culture in malaria - two normal cell populations with one patient cell population

Assay #	Normal Responder Cells				Patient Responders	
	A + Bm	A + Cm	B + Am	B + Cm	C + Am	C <sub>m</sub> + Bm
1	2.6*	1.3	3.3	3.8	1.8	2.1
2	3.8	2.5	2.6	2.7	1.8	3.1
3	12.6	3.7	4.4	3.6	10.3	20.2
4	14.1	8.0	13.3	9.0	5.1	3.2
5	7.0	2.4	6.4	9.2	2.8	5.5
6	2.1	2.5	1.6	6.8	15.4	8.1
7	10.9	2.0	3.9	2.6	2.0	4.0
8	2.6	3.2	5.6	1.2	10.0	12.0
9	1.4	-	1.4	1.1	2.2	38.2
10	16.6	8.0	1.5	5.2	1.3	6.2
Mean :	7.0	3.4	5.3	5.9	6.2	7.0

\* Stimulation index

A = Normal cells

B = Normal cells

C = Patient cells

X<sub>m</sub> = Mitomycin treated cells

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