

Transformation of Patient Lymphocytes by Selected Malarial Antigens

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OBJECTIVE : To develop methodology for the specific stimulation of malaria patient lymphocytes by erythrocyte and sporozoite antigens.

BACKGROUND : Several assays have been developed which describe specific *in vitro* responsiveness of host lymphocytic cells to stimulation by malaria antigens. These assays have employed either rodent (1, 2) or human material (3). This report summarizes studies utilizing related techniques, with cells from adult Thais infected with either *P. falciparum* or *P. vivax*.

METHODS : Mononuclear leukocytes were cultured in modified RPMI 1640 media according to the methodology of MacDermott *et al.* (4). Assays for lymphocytes from falciparum infections utilized patient lymphocytes and falciparum antigen extracted from either infected chimpanzee erythrocytes or sporozoites from human infections. Controls were uninfected cells and media. Cell suspensions were incubated 6 days at 37°C in 5% CO₂. Lymphocyte concentrations were either 3 x 10⁵ or 4 x 10⁵ cells per well with 1 ug of erythrocytic antigen or 1.42 ug₃ of sporozoites. After initial incubation, cultures were pulsed with 0.4 uci ³H thymidine for 24 hours. The lymphocytes were then processed by a multiple automated sample harvester (MASH). After drying, filter pads containing the cells were placed in scintillation vials containing hydromix and counts (CPM) were determined in a Hewlett-Packard beta scintillation counter. Stimulation indices (SI) of patient lymphocytes were calculated by the following formula :

$$SI = \frac{\text{CPM test}}{\text{CPM control}}$$

RESULTS : Table 1 illustrates the mean counts and stimulation indices of twelve Patients infected with *P. falciparum*. These results are similar to those of 9 other falciparum patients at the same cell concentration (4 x 10⁵ cells/well) under optimal conditions and to results of 9 additional falciparum patients whose cell concentration was 1 log lower under the same conditions of incubation. In comparing the results of these patients with those of normal donors (Table 2), there are no apparent differences. The range and mean values for both the counts and stimulation indices are essentially identical. Similar results were likewise observed when patient cells (Table 3) or normal cells (Table 4) were stimulated with falciparum sporozoite antigens. It is clear that definitive studies in both trophozoite and sporozoite induce transformation must await improved antigenic material through increased antigen concentration (by culture) and improved purification of human material. It is likewise imperative that the variables of parasite concentration and duration of infection be controlled to the greatest

extent possible by careful patient selection in future work. Pending the constraint of these variables this research should be considered complete. This is a final report.

Table 1. Response of malaria patient lymphocytes to stimulation with *P. falciparum* antigen

(PF) Patient #	% Monocytes	Counts Per Minute	Stimulation Index
099	13	7859	2.5
104	14	3806	2.3
108	11	17021	4.6
118	10	552	5.9
130	14	2868	1.1
137	13	4037	2.9
134	9	5942	5.0
140	12	8731	3.9
142	13	10730	23.6
146	12	1735	3.1
149	10	3384	14.5
152	8	6907	24.4
Range :	8-14	552-17021	1.1-24.4
Mean	11.6	6131	7.8

Table 2. Response of normal lymphocytes to stimulation with *P. falciparum* antigen (Chimpanzee preparation) - Initial and follow up values

Donor	Initial CPM/S.I.		CPM/S.I. at 24 Hours		CPM/S.I. at 1 Week	
Turien (M)	7127	14.9	4319	13.2	12554	23.9
Somchai (F)	273	1.2	-	-	-	-
Prigg (M)	3699	14.1	8069	25.2	12072	9.0
Prayote (M)	8643	3.6	-	-	-	-
Prasert (M)	13399	1.9	-	-	-	-
Sanei (M)	9494	15.9	2507	5.4	9432	22.5
Prasit (M)	8689	15.1	5845	11.0	10916	21.6
Boontum (M)	14595	20.0	-	-	-	-
Komson	8553	17.5	-	-	-	-
Udorn (M)	4870	7.8	-	-	-	-
Sitt (M)	4535	5.2	-	-	-	-
Kaew (M)	13687	17.0	14979	7.6	9705	6.2
Boonkum (M)	9019	5.5	-	-	-	-
Boonmee (M)	11712	9.0	-	-	-	-
Barnyen (F)	12838	11.1	-	-	-	-
Somchit (F)	247	2.4	-	-	-	-
Range :	247-14595	1.9-20.0	2507-14979	5.4-25.2	9432-12554	6.2-23.9
Mean :	8211	10.1	7144	12.5	10936	16.6

Table 3. Response of lymphocytes from *P. falciparum* patients to stimulation with *P. falciparum* sporozoite antigen (1.42 ug/well)

Patient #	% Monocyte	Counts Per Minute	Stimulation Index
106	10	813	1.4
108	11	4076	1.7
134	10	2106	1.2
138	10	456	2.7
149	10	518	2.7
152	8	2494	10.1
153	14	1347	4.1
Range :	8-14	456-4076	1.2-10.1
Mean :	10.4	1687	3.4

Table 4. Response of Normal lymphocytes to stimulation with *P. falciparum* sporozoite antigen (1.42 ug/well)

Donor	% Monocytes	Counts Per Minute	Stimulation Index
Barnyen	12	4047	13.4
Boonmee	13	582	2.0
Somboon	11	6103	2.8
Niphon	12	6563	1.9
Sanei	10	2569	3.9
Range :	10-13	582-6563	1.9-13.4
Mean :	11.6	3973	4.8

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