Mitogen Responsiveness of Lymphocytes from Patients Infected with Malaria

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OBJECTIVE : To compare the responsiveness of malaria patient lymphocytes to stimulation by selected plant mitogens with responsiveness of normal lymphocytes.

BACKGROUND : The application of plant mitogen stimulation in the study of cellular immunology has been well documented. This methodology has been most often utilized in the study of T lymphocytes but has also been employed in investigations of B lymphocyte activation (1). The salient feature of this approach is the assessment of mitogen mediated activation by blastogenesis or by enhanced uptake of radio labelled amino acids. These techniques were employed in the activation of malaria patient lymphocytes using the plant mitogens phytohemagglutinin (PHA), Concanavalin A (CON A), and Pokeweed mitogen (PWM).

METHODS : Mononuclear cells were isolated by ficoll hypaque centrifugation according to the methodology of Boyum (2). The cells were then washed in Selegmans balanced salt solution (SBSS) and further processed according to the methodology of Chess et al. with modification (3). Each aliquot of cells was adjusted to 1.5×10^6 cells/ml in RPMI 1640 media in flat bottom microtiter plates. Each assay was conducted in a set of 10 wells. Mitogens were added in the following concentrations per microtiter well : PHA, 5 ug; CON A, 2 ug; PWM, 25 ug. Cultures were incubated for 72 hours in 5% CO_2 and were pulsed with 0.4 uCi ^{3}H-thymidine for 24 hours. Samples were harvested with a multisample harvesting machine and washed with distilled water. Dried filter paper discs were then transferred to scintillation vials and suspended in Hydromix before counting in a Hewlett-Packard beta counter. The mean value for the counts per minute (CPM) and the standard deviation of the mean were determined. The stimulation index (S.I.) was determined for each sample. The S.I. was calculated with the following formula : S.I. = mean test CPM/mean control CPM.

RESULTS : Table 1 summarizes the PHA response of patient lymphocytes as compared with that of uninfected volunteers. The response of patient lymphocytes is not significantly different from that of the normal controls (mean values 60,954 vs 59,897 CPM respectively). The S.I. results suggest a lower PHA stimulation of patient lymphocytes. These results are, however, somewhat misleading since the S.I. is highly influenced by variation in the unstimulated control cultures. In a few assays the CPM in the control cultures were excessively high and the corrected CPM therefore gives more reliable data. Responsiveness of patient lymphocytes to stimulation with CON A (Table 2) was significantly lower than normal lymphocytes. Mean CPM values were 23,260 vrs 28,880 respectively while values for the S.I.'s were 38.1 and 43.0. The PWM response

(Table 3) indicates similar results for patients and controls. The mean CPM values were 29,548 for patients and 28,612 for controls while the S.I.'s were 44.4 and 42.2. The overall conclusions from this study are that responsiveness of patient lymphocytes to PHA and PWM are normal. The responsiveness to CON A appears slightly depressed and may be attributed to a missing T cell population as has been reported elsewhere. A manuscript concerning this work is in preparation. This project is complete.

REFERENCES :

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Table 1. PHA Response of Human Lymphocytes Normal vs Patients with Malaria

Type of Infection	CPM		S.D.		S.I.	
	Patient	Control	Patient	Control	Patient	Control
P.V.	80318.67	47164.67	4231.40	5888.81	101.56	134.73
P.F.	63722.94	56851.33	10101.02	6167.80	62.76	36.26
P.V.	60211.50	70402.00	4787.71	18530.64	63.82	76.49
P.V.	81716.67	50268.07	8292.28	4197.17	60.69	51.69
P.V.	74686.34	44581.66	5788.40	2691.16	62.23	56.73
P.V.	53735.50	88429.34	8424.37	13515.63	43.45	198.53
P.V.	60946.83	64727.85	8676.35	8348.38	40.80	56.44
P.F.	65889.83	58501.66	3554.21	9310.35	85.09	172.05
P.F.	52528.40	49503.40	4525.07	2711.54	61.03	95.83
P.F.	47506.08	48450.66	2734.23	4532.59	91.53	71.21
P.V.	71673.00	51913.83	4202.38	3039.66	158.26	68.24
P.V.	59595.06	66697.40	8646.78	5089.15	145.97	38.30
P.F.	44783.17	60399.33	2379.39	3346.25	88.07	106.16
P.V.	47700.15	69055.83	3014.45	10162.36	141.45	119.95
P.F.	49309.60	71516.50	3462.36	7835.34	56.59	117.22
Mean	60954.91	59879.66	5521.36	7024.45	84.22	93.32

Table 2. Con-A Response of Human Lymphocytes Normals vs Patients with Malaria

Type of Infection	CPM		S.D.		S.I.	
	Patient	Control	Patient	Control	Patient	Control
P.V.	21437.00	16993.61	3975.83	5376.43	27.84	49.18
P.F.	14648.34	32894.67	1590.26	6845.42	15.19	21.40
P.V.	23202.50	40032.67	4211.39	4554.16	25.21	43.93
P.V.	15116.00	28399.23	2886.33	4616.20	12.04	29.63
P.V.	34532.80	20278.66	5279.55	1784.14	29.31	26.09
P.V.	23545.67	27345.17	4163.42	4632.56	19.60	62.08
P.V.	34098.17	41888.26	6380.89	6321.78	23.26	36.88
P.F.	28210.50	24325.66	2012.72	4056.54	37.00	72.12
P.F.	24900.17	31101.33	3900.19	6767.93	29.45	60.58
P.F.	28724.91	20315.83	2946.31	4778.17	55.74	30.44
P.V.	34163.75	23889.25	2919.29	3687.85	75.96	31.94
P.V.	37352.40	32336.53	2541.00	1608.86	91.86	19.08
P.F.	19978.17	33591.67	1358.22	3641.95	39.84	59.48
P.V.	20168.06	29772.66	3987.32	5592.75	60.38	52.28
P.F.	24912.50	30037.83	2702.04	5082.04	29.08	49.81
Mean	23260.37	28880.20	3390.31	4623.11	38.11	42.99

Table 3. PWM Response of Human Lymphocytes Normals vs Patients with Malaria

Type of Infection	CPM		S.D.		S.I.	
	Patient	Control	Patient	Control	Patient	Control
P.V.	25034.00	14201.74	3217.01	4867.75	32.34	41.27
P.F.	30940.34	24425.50	2669.25	2832.52	30.99	16.15
P.V.	29286.83	57325.75	3706.30	10466.97	31.36	62.47
P.V.	14972.00	22794.73	2049.15	1938.88	11.93	23.98
P.V.	30310.51	23293.16	3828.76	1906.73	25.85	29.82
P.V.	33154.17	17125.50	4684.42	2571.13	27.19	39.25
P.V.	37537.67	55397.26	6696.60	5724.47	25.51	48.45
P.F.	32463.83	31008.00	2060.85	3082.87	42.43	91.66
P.F.	16774.75	22359.50	1022.40	3489.70	20.17	43.88
P.F.	27369.58	19456.33	3328.38	1529.76	53.15	29.19
P.V.	45750.00	23399.83	4132.98	2637.83	101.38	31.31
P.V.	46545.90	25248.53	4056.37	1949.80	116.61	15.12
P.F.	21192.50	29701.33	3357.35	2091.85	42.20	52.71
P.V.	24084.06	25357.50	4125.27	5947.58	71.91	44.75
P.F.	27812.16	38099.83	4450.02	4153.85	32.35	62.91
Mean	29548.55	28612.23	3559.00	3679.44	44.37	42.19