Effect of Sera from Malaria Patients on Mitogen Responsiveness of Normal Lymphocytes

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

Associate Investigators : Somchai Kokchareon
Theera Wimonwattrawatee

OBJECTIVE : To evaluate the effect of sera from patients infected with falciparum or vivax malaria on the responsiveness of lymphocytes to stimulation with selected plant mitogens.

BACKGROUND : Stimulation of human cells with plant mitogens has been well documented (1). In this approach, nonspecific stimulation is evaluated by cellular blastogenesis or by the enhanced uptake of radiolabeled amino acids. In the present studies it was decided to search for a possible suppressive effect of pooled malaria patient sera against the responsiveness of normal lymphocytes to stimulation with 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 washed in Selegmans balanced salt solution (SBSS) and were further processed according to the methodology of Chess *et al.* with modification (3). Test suspensions were made by mixing 5 ml of pooled patient (malaria species specific) sera with 20 ml of RPMI 1640 media. Similar 20% suspensions were made for control preparations in which pooled normal sera were used. Normal mononuclear cells were adjusted to concentrations of 1.5×10^6/ml with either of the above suspensions. Mitogen suspensions were likewise made up with these sera/media mixtures to the following concentrations : PHA 50 ug/ml, CONA 20 ug/ml and PWM 250 ug/ml. A 0.1 ml volume of the respective mitogen suspension was added to each microtiter plate well along with 0.1 ml of cell suspension. Stimulation controls were comprised of cells, sera and media without mitogen. Cultures were incubated for 72 hours in 5% CO_2 and were pulsed with 0.4 uCi ^{3}H-thymidine for 24 hours. Cell samples were harvested onto filter paper discs and transferred to scintillation vials. After suspension in hydromix, counts were performed in a Hewlett-Packard beta counter and both counts per minute and stimulation indexes were determined.

RESULTS : Table 1 summarizes the effect of patient sera on the responsiveness of normal cells to mitogenic stimulation. Significant suppression was seen where patient sera from either source was admixed. This was true regarding both responsiveness to PHA and CONA which stimulate T lymphocytes. On the other hand, significant suppression did not result in the cellular response to PWM - a mitogen which predominantly effects B lymphocytes. A manuscript is in preparation concerning this work and that involving responsiveness of patient lymphocytes to these same mitogens. This is a final report.

Table 1. Effect of malaria patient sera on lymphocyte response to mitogens

Serum Source	Phytohemagglutinin (PHA)		Concanavalin A (CONA)		Pokeweed Mitogen (PWM)	
	Normal	Malaria	Normal	Malaria	Normal	Malaria
	138	62+	92	46	53	66
	102	69+	81	45	46	60
	102	75*	100	40	57	91
	148	64+	105	47	63	41
	99	37*	72	11	49	20
	206	49*	83	34	44	48
	181	128+	63	46	26	124
	181	81*	63	16	26	46
	191	61*	148	72	100	100
	191	45+	148	59	100	78
	101	80+	66	68	36	79
	101	87*	66	23	36	13
	151	79+	144	60	73	79
	151	111*	144	43	73	54
	190	72+	142	57	111	75
	124	47+	79	54	50	68
	131	61+	132	116	139	183
	161	105+	153	165	121	159
	192	181+	112	101	45	29
	145	59+	80	76	30	107
Range :	99-206	37-181	63-153	11-165	26-139	13-159
Mean :	149	78	104	59	65	76
			PF Sera Data (13 Assays)			
Range :	101-192	45-181	63-152	45-165	26-139	29-182
Mean :	150	81	108	72	69	88
SD	31	38	33	35	37	44
P	0.001		0.02		0.3	
			PV Sera Data (7 Assays)			
Range :	99-206	36-111	63-148	11-72	26-100	20-100
Mean :	147	72	97	34	55	53
SD	47	25	36	21	25	33
P	0.005		0.005		0.95	

\+ *P. falciparum* Serum

* *P. vivax* Serum

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