

## Glucosamine (<sup>14</sup>C) Uptake in Malaria Activated Human Macrophages

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

Associate Investigators : Somchit Tulyayon  
Prasit Sookto  
Niphon Chuanak

**OBJECTIVE :** To evaluate the activation of monocytes by lymphokines synthesized by malarious lymphocytes as determined by uptake of radiolabeled (<sup>14</sup>C) glucosamine.

**BACKGROUND :** The mechanisms of human white cell response to malaria are poorly understood. Based on work by other investigators (1) a series of investigations were undertaken to evaluate the activation of macrophages through radioimmuno-technique. This is a report of these studies.

**METHODS :** The assays were conducted with modifications of the methodology of Hammond and Dvorak (2). Mononuclear cells were isolated by ficoll hypaque centrifugation and adjusted to a concentration of  $1.5 \times 10^6$  monocytes per culture. Test antigens were either extracts of pooled *P. vivax* blood forms (human source) or *P. falciparum* (chimpanzee source). Antigen concentrations were either undiluted or diluted 1:2. Positive controls consisted of cells with the plant mitogen phytohemagglutinin. Negative controls were cells and RPMI 1640 media only. Lymphokines were synthesized either with all cells present or with lymphocytes absent. In the latter case, nonadherent cells were decanted after 2 hours and media replaced to the original volume. Lymphokines were synthesized and set up as for the previously reported (Lowry) assay. Normal cell cultures were incubated with lymphokines in 5% CO<sub>2</sub> for 72 hours. The cultures were next pulsed with 5 uCi of glucosamine and incubation was continued for 6 hours. Each monolayer was then washed with cold Hank's buffer solution containing non-radio-labeled glucosamine. Vials were drained and frozen at -20°C until required. Each vial was thawed and treated with cold 10% TCA with calf serum and stored at 40°C for 1 hour. After centrifugation the supernatant was decanted and hydromix added to precipitate the radiolabeled proteins. Counts were performed in a Hewlett-Packard beta counter.

**RESULTS :** Table 1 summarizes radioisotope uptake when patient cells were incubated with concentrated or diluted falciparum antigen in the presence or absence of lymphocytes. While the mean SI values for these experiments were acceptable, these results were marred by 8 negative SI values including 4 in the PHA control. Evaluation was further clouded by low CPM values—often only a few CPM's above those of background correction. Similar findings are recorded for *P. vivax* incubations as seen in Table 2. It was concluded that the potential usefulness of this assay is dependent on concentration and purification of the malaria antigen and standardization of techniques. This assay has been discontinued in

favor of those of higher priority. This is a final report.

Table 1. Glucosamine ( $^{14}\text{C}$ ) uptake in cells incubated with *P. falciparum* antigen

Lymphocytes Present

Assay No.	<u>Concentrated Antigen</u>			<u>Diluted Antigen</u>			<u>PHA</u>		
	Control	Test	SI	Control	Test	SI	Control	Test	SI
1	300*	236	-	328	345	1.1	275	232	-
2	21	16	-	18	25	1.4	18	22	1.2
3	33	63	1.9	15	76	5.1	28	59	2.1
4	25	75	3.0	25	75	3.0	25	59	2.4
5	42	255	6.1	36	1050	29.1	949	455	-
6	18	94	5.2	28	47	1.7	15	124	8.3
Mean SI+ :			4.1	6.9			12.3		

Lymphocytes Absent

Assay No.	<u>Concentrated Antigen</u>			<u>Diluted Antigen</u>			<u>PHA</u>		
	Control	Test	SI	Control	Test	SI	Control	Test	SI
1	33	179	5.4	57	74	1.3	170	119	-
2	4	52	13	153	77	-	317	1499	4.6
3	6	10	1.6	11	18	1.6	76	298	3.9
4	121	55	-	17	83	4.9	631	522	-
Mean SI :			6.6	2.6			4.3		

\* Net counts per minute

+ Stimulation Index - negative values eliminated from calculations

Table 2. Glucosamine ( $^{14}\text{C}$ ) uptake in cells incubated with *P. vivax* antigen

Lymphocytes Present

Assay No.	Concentrated Antigen			Diluted Antigen			PHA		
	Control	Test	SI	Control	Test	SI	Control	Test	SI
1	446	462	1.1	134	5676	42.3	1417	745	-
2	30	12	-	45	173	3.8	34	33	-
3	15	218	14.5	17	20	1.2	15	170	11.3
Mean SI* :			7.8	15.8			11.3		

Lymphocytes Absent

Assay No.	Concentrated Antigen			Diluted Antigen			PHA		
	Control	Test	SI	Control	Test	SI	Control	Test	SI
1	67	815	12.2	92	140	1.5	96	278	2.9
2	27	109	4.0	68	92	1.4	27	185	6.9
3	214	79	-	42	52	1.2	225	253	1.1
Mean SI* :			8.1	1.4			3.6		

\* Negative SI's eliminated from calculations

REFERENCES :

1. Rosenthal, A.S., Lipsky, P.E. and Shevach, E.M. Macrophage-Lymphocyte Interaction and Antigen Recognition. Fed. Proc. 34:1743-1748, 1975.
2. Hammond, M.E. and Dvorak, H.F. Antigen - Induced Stimulation of Glucosamine Incorporated by Guinea Pig Peritoneal Macrophages in Delayed Hypersensitivity. J. Exp. Med. 136:1518-1532, 1972.