

## Lymphokine Mediated Macrophage Activation as Demonstrated by Enhanced Protein Synthesis

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**OBJECTIVE :** To evaluate lymphokine mediated macrophage activation in malaria patients through the parameter of increased protein synthesis (Lowry technique).

**BACKGROUND :** The work of Criswell and her colleagues (1) supports the notion that humoral factors, possibly other than antibody, may activate macrophages during the malaria infection. Workers using other immunological systems have provided insight into macrophage activation (2, 3). This report summarizes data relative to macrophage activation in cases of human malaria.

**METHODS :** These assays were conducted according to the methodology of Nathan (4). In synthesizing lymphokines,  $2 \times 10^6$  patient mononuclear cells were incubated with either undiluted chimpanzee source falciparum antigen (WR 352 low) or with ammonium chloride extracted vivax antigen (human source) for 48 hours. Cultures were centrifuged and supernatants frozen until required. Control supernatants were prepared by incubating patient cells with media only, adding antigen immediately prior to the centrifugation step. Additional controls were culture supernatants from patient cells with normal human red cell extract or normal cells with antigen. For the adherence assay (Lowry technique) culture supernatants were incubated with normal mononuclear cells for 3 days. After decanting the supernatants, the monolayers were treated with NaOH and Lowry protein concentrations were determined.

**RESULTS :** Table 1 summarizes the results of 14 assays on lymphokine activity from falciparum sources with antigen. These are compared with 4 assays each for patient cells with normal red cell extract and normal white cells with malaria antigen. There resulted a mean protein value of 126  $\mu\text{g/ml}$  for the patient cells with antigen compared with 100  $\mu\text{g/ml}$  for the cell control and 63  $\mu\text{g/ml}$  for the antigen control. These results suggest specific activity in lymphokines synthesized by the patient white cells in the presence of antigen. Table 2 displays the results of 15 assays from vivax patient cells as compared with 4 assays each with the cell and antigen control. Here the mean value for the cell control (142  $\mu\text{g/ml}$ ) was higher than that for the test system (93  $\mu\text{g/ml}$ ). This implies that where a crude extract of human antigen is used, test and control group activity may be comparable. Alternately, the enhanced results in the falciparum system could be an artifact. This assay is clearly of value in future research; however, concentration with purification of malaria antigen will be necessary. Because of higher immediate priorities in other areas these assays have been discontinued. This is a final report.

Table 1. Lymphokine mediated macrophage activation in malaria - *P. falciparum*

Lymphokines from Patient Cells with Malaria Antigen

<u>Patient #</u>	<u>Control</u>	<u>Test</u>	<u>Increase (µg/ml)</u>
96	62	158	96
100	129	220	91
105	82	175	93
109	40	112	72
122	101	337	236
144	80	211	131
154	90	133	42
161	160	310	150
164	175	325	151
175	242	213	(-)29
187	126	120	(-) 6
189	199	475	276
190	263	417	154
191	153	245	245

Range : (-)29-276  
Mean : 126

Lymphokines from Patient Cells with Normal Red Cells

<u>Patient #</u>	<u>Control</u>	<u>Test</u>	<u>Increase (µg/ml)</u>
114	84	160	73
151	117	172	56
167	95	210	116
170	219	226	7

Range : 7-116  
Mean : 63

Lymphokines from Normal Cells with Malaria Antigen

<u>Donor</u>	<u>Control</u>	<u>Test</u>	<u>Increase (µg/ml)</u>
Somchai	101	167	66
Barnyen	117	216	99
Turien	57	219	72
Prasit	177	340	162

Range : 66-163  
Mean : 100

Table 2. Lymphokine mediated macrophage activation in malaria - *P. vivax*

Lymphokines from Patient Cells with Malaria Antigen

<u>Patient #</u>	<u>Control</u>	<u>Test</u>	<u>Increase (µg/ml)</u>
102	69	317	249
117	18	111	93
125	72	111	40
123	128	149	20
129	81	194	113
131	74	132	58
133	65	292	227
135	168	199	31
139	32	82	50
141	150	206	56
148	188	213	25
150	209	247	138
156	169	321	161
158	130	179	48
184	276	365	89

Range : 31-249  
Mean : 93

Lymphokines from Patient Cells with Normal Red Cells

<u>Patient #</u>	<u>Control</u>	<u>Test</u>	<u>Increase (µg/ml)</u>
155	180	148	(-) 32
163	74	189	115
166	94	91	(-) 3
168	140	196	59

Range : (-) 32-115  
Mean : 35

Lymphokines from Normal Cells with Malaria Antigen

<u>Donor</u>	<u>Control</u>	<u>Test</u>	<u>Increase (µg/ml)</u>
Barnyen	189	303	113
Prasit	234	295	62
Katchrinnee	115	382	266
Prayote	130	257	127

Range : 62-266  
Mean : 142

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