Malaria Patient Lymphokine Mediated Macrophage Activation as Demonstrated by Enhanced Adherence and Protein Synthesis

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OBJECTIVE : To develop an assay to describe lymphokine mediated activation of macrophages by the parameters of increased macrophage adherence and protein synthesis (Lowry).

BACKGROUND : Data by Criswell and her colleagues (1) confirms earlier notions that humoral factors, possibly other than antibody, may activate macrophages in the destruction of the malaria parasite. Although research in this area is just now being started by malariologists, workers in other field have provided such insight through related systems (2, 3). This report summarizes preliminary data in an effort to apply the theory and technology of these studies to investigations of the human response to malaria.

METHODS : The assays conducted were accomplished according to the methodology of Nathan and his colleagues (4). In synthesising lymphokines, 2 x 10^6 patient mononuclear cells were incubated with *P. vivax* antigen either full strength or diluted 1:2 for 48 hours. Cultures were then centrifuged and supernatants frozen until required. Control supernatants were prepared by incubating patient cells with media only, adding antigen immediately prior the centrifugation step. For the adherence assay in the presence of lymphocytes supernatants were incubated with normal mononuclear cells for 3 days. After decanting the supernatants monolayers were treated with NaOH and Lowry protein concentrations were determined. An adherence assay was also made in the absence of lymphocytes; for this all steps outlined above were accomplished except that the supernatant containing non-adherent lymphocytes was decanted after 2 hours followed by washing in hepes buffer before the 3 day incubation was begun.

RESULTS : Table 1 summarizes the results of macrophage adherence with lymphocytes present. The test series shows an overall mean value of 77 ug/ml protein vs 56 ug for the control group. Data in Table 2 suggests a similar pattern with mean values of 59 and 34 for test and control groups respectively. The preliminary data in both of these assays suggests an enhancement in macrophage activation mediated by antigen stimulated lymphokine synthesis. These assays seem encouraging and serve as the basis for continuing studies.

Table 1. Lowry Protein Values (ug/ml) - Lymphocytes Present

Specimen No.	Test High/Low Initial Ag Conc.	Test Mean	Control
1	119.5 101.1	110.3	61.8
2	82.5 80.3	81.4	95.8
3	65.6 86.2	75.9	47.9
4	39.0 42.0	40.5	18.0
Overall Mean		77.0	55.9

Table 2. Lowry Protein Values (ug/ml) - Lymphocytes Absent

Specimen No.	Test High/Low Initial Ag Conc.	Test Mean	Control
1	79.5 85.0	82.3	33.3
2	74.4 57.5	66.0	33.2
3	82.5 60.4	71.5	45.0
4	12.0 21.0	16.5	25.5
Overall Mean		59.0	34.3

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