Synthesis of Lymphocyte Blastogenic Factor by Sensitized Lymphocytes from Malaria Patients

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OBJECTIVE : To develop methodology for the activation of normal lymphocytes by lymphokines from antigen stimulated by lymphocytes from malaria patients.

BACKGROUND : These studies were undertaken in an attempt to determine whether certain mechanisms of immune activation parallel those in better defined systems. There is considerable evidence in these systems that normal lymphocytes undergo blast transformation and incorporate ^{3}H thymidine when cultured in the presence of supernatants from lymphocytes stimulated with antigen (1, 2). The synthesis of such blastogenic factors has been associated with T lymphocytes and is a measure of cell mediated immunity. The studies reported here represent attempts to induce blastogenesis by factors produced by specifically stimulated lymphocytes from malaria patients.

METHODS : Mononuclear leukocytes from *P. falciparum* patients were isolated by ficoll-hypaque centrifugation. The cells at concentrations of either 1 x 10^6/ml or 3 x 10^6/ml were incubated with falciparum antigen from infected chimpanzee erythrocytes for 48 hours at 37°C in 5% CO_2. Following centrifugation, supernatants were frozen until required. Control preparations were supernatants exposed to antigen immediately before centrifugation. For the remainder of the assay, non-sensitized (normal) lymphocytes were incubated with the supernatants for 6 days. Cultures were then pulsed with 0.4 uCi ^{3}H thymidine before incubating another 24 hours. The cells were isolated and washed in a multiple automated sample harvester (MASH). After drying on filter pads the cellular material was placed in a scintillation vial with hydromix. Counts for radioactivity (CPM) were conducted on a Hewlett-Packard beta counter.

RESULTS : Table 1 summarizes the results from assays on the blastogenic activity of supernatants from 19 isolates of patient cells. The net counts for these assays ranged between 506 and 3629 (mean 1878). The stimulation indices (SI) for these counts (mean 4) met or exceeded those generally in the literature for similar studies. An attempt was made to increase the stimulation indices by increasing the concentration of lymphokine producing patient cells three fold to 3 x 10^6 cells/ml. The results summarized in Table 2 indicate that this attempt was not successful since both the range and mean of the SI values in 6 assays were lower than when the cells were at lower concentrations. It became clear through these experiments that marked blastogenesis results from lymphokines raised by incubating patient lymphocytes with falciparum antigen. Future consideration of this assay in further immunologic analysis is therefore warranted.

The evaluation of the present experimental series is, however, made difficult because of the chimpanzee origin of this antigen and by the lack of a normal chimpanzee red cell control. Definitive work with this assay must await the concentration and purification of human falciparum antigen and appropriate control material. This is a final report.

Table 1. Blastogenesis - lymphokines from 1 x 10^6/ml *P. falciparum* lymphocytes with normal cells

Assay #	Mean CPM	S.I.
1	7879	1.5
2	1334	4.3
3	1405	1.3
4	506	2.1
5	912	1.9
6	1772	1.9
7	767	3.1
8	549	2.2
9	1114	5.4
10	961	4.7
11	1485	5.6
12	939	3.5
13	3629	5.9
14	3247	5.3
15	817	4.0
16	1202	5.9
17	3007	3.6
18	1988	6.8
19	2176	7.4
Range :	506-3629	1.3-7.4
Mean :	1878	4.0

Table 2. Blastogenesis - lymphokines from 3 x 10^6/ml *P. falciparum* lymphocytes with normal cells

Assay #	Mean CPM	S.I.
1	3332	4.9
2	2305	2.1
3	966	1.9
4	1796	3.3
5	621	1.3
6	3445	5.4
Range :	621-3445	1.3-5.4
Mean :	2076	3.2

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