

Synthesis of Lymphocyte Blastogenic Factor by Sensitized Lymphocytes from Malaria Patients

Principal Investigators : Robert A. Wells, MAJ, MSC
Sandor Zolyomi, SFC, USA
Pirom Phisphumvithi

Associate Investigators : Somchai Kokchareon
Theera Wimonwatrawatee

OBJECTIVE : To develop preliminary methodology for the activation of normal lymphocytes by lymphokines induced by malaria antigens as a basis for more definitive studies.

BACKGROUND : While it may be assumed that the mechanisms of immunologic activation in malaria parallel those of better studied systems, at present few studies have been accomplished to support or refute this assumption. One feasible parameter of exploration is the application of lymphokines elicited through the interaction of antigens with specifically sensitized lymphocytes. There is a considerable body of evidence that non-sensitized lymphocytes undergo blast transformation and incorporate ³H thymidine when cultured in the presence of supernatants from such stimulated lymphocytes (1, 2). The synthesis of the blastogenic factor(s) is dependent upon T lymphocytes as opposed to B lymphocytes and is considered to be a manifestation of cell mediated immunity. The studies reported here are preliminary to more definitive investigations of activation mechanisms in malaria.

METHODS : Mononuclear leukocytes from *P. falciparum* patients were isolated by ficoll-hypaque centrifugation. The cells were incubated with falciparum antigen from infected chimpanzee erythrocytes for 48 hours at 37°C in 5% CO₂. 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 lymphocytes were incubated with the supernatants for 6 days. Cultures were then pulsed with 0.4 uCi ³H 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 containing 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 3 malaria patients. Although the differences between test and control values are small, they are within the range of results from earlier workers using other systems (2). It is clear, however, that further standardization toward optimal conditions is in order. Alterations of cell concentration, type and concentration of antigen and incubation time are planned. This project continues.

Table 1. Results from Preliminary Studies on Blastogenic Activity from Malaria Patient Lymphokines

Assay #	Test (CPM)	Control (CPM)
1	1332	694
2	1405	1115
3	911	439
Range	911 - 1332	439 - 1115
Mean	1216	749

REFERENCES :

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