

Glucosamine Uptake in Malaria Activated Human Macrophages

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OBJECTIVE : To develop an assay to describe the activation of monocytes through interaction with malarious lymphocytes as determined by increased uptake of radiolabelled glucosamine (^{14}C glu).

BACKGROUND : The mechanisms of host response to malaria infection at the cellular level are poorly understood at present. Findings by other workers have provided a basis for pursuing basic investigations in this area (1). It was reasoned that techniques could be developed to evaluate activation of mononuclear cells through radioimmunotechnique. This report describes preliminary results from these studies.

METHODS : The assays conducted were modifications of the methodology of Hammond and Dvorak (2). Mononuclear cells were isolated by ficoll-hypaque centrifugation and adjusted to a concentration of 1.5×10^6 monocytes per culture. Test antigens consisted of either pooled *P. vivax* merozoite or sporozoite antigens. Positive controls consisted of cells and PHA mitogen, negative controls were cells and RPMI 1640 media only. Each cell culture was incubated in 5% CO_2 for 72 hours. The cultures were then pulsed with 5 uCi of glucosamine with continued incubation for 6 hours. Each monolayer was washed with cold Hank's containing non-radiolabelled glucosamine. Vials were drained and frozen until required. For the conduct of the assay, the contents of each vial were treated with cold 10% TCA containing calf serum and stored at 4°C 1 hour. After centrifugation the supernatant was decanted and Hydromix was added to precipitate the radiolabelled proteins. Counts were performed in a Hewlett-Packard beta counter.

RESULTS : The results of a single assay completed are unremarkable. The positive (PHA) control indicated stimulation as expected but the antigen - test values resembled those of the negative control. The development of this assay continues.

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.