

Cryogenic Preservation of Malaria Lymphocytes

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OBJECTIVE : To develop the capability for freezing and long-term storage of lymphocytes isolated from the blood of individuals with malaria.

BACKGROUND : Numerous immunological studies have been performed to investigate the humoral response of humans to malaria infection (1, 2) while relatively few studies have investigated the role lymphocyte subpopulations may have in either the protection or recovery of individuals from naturally acquired malaria infections.

A major reason for this difference is that the cells must be processed and used quickly, unless facilities are available for controlled freezing. Serum can be processed and stored with a minimum of time and equipment.

For the last several years this laboratory has been engaged in cellular immune studies using lymphocytes from patients with naturally acquired malaria. Although progress has been made, two characteristics of the epidemiology of the disease have hampered previous studies.

First, because the majority of malaria cases are found in rural areas, collection teams are limited in the number of blood samples that they can obtain, screen, process, and transport each morning. They must allocate enough time to ensure that the processed samples are returned to our laboratory, by late afternoon, for use in assays. Any patients who arrive at the field treatment site after a designated "cut off" time are not bled for cell studies.

Secondly, we are restricted by the seasonal occurrence of the disease. The maximum number of cellular assays are performed during the "peak" season; however, few malaria cellular assay can be performed during the rest of the year.

A controlled cell freezing capability will remove some of the limitations mentioned above and will enable us to:

1. Set up an "intermediate" laboratory facility closer to the blood collection sites where blood can be processed and cells frozen for temporary storage. This will decrease transportation time and will allow technicians to collect blood from volunteers throughout the day. Once the blood is collected it can be transported a short distant and processed and stored at the "intermediate" lab before being transported back to AFRIMS.

2. Collect, freeze, and store patient lymphocytes for use during the months when the incidence of malaria is lower, so that cellular research experiments can continue throughout the year.

3. Begin sequential *in vitro* studies of the cellular immunocompetence of malaria patients. These studies previously have been limited by day-to-day test variability, making interpretation of results difficult. Several authors have advocated the use of frozen lymphocytes in these *in vitro* assays to reduce day-to-day test variation, thereby enabling a sequential set of lymphocytes from a patient to be thawed and tested on the same day (3, 4).

METHODS : Cryogenic equipment will be set-up and standardized at AFRIMS before being moved to an acceptable "intermediate" facility.

The Biological Freezing System (Union Carbide Corp. Linde Division, N.Y., N.Y.) to be used includes:

1. A BF-4 rate controller
2. A BF-4-1 freezing chamber
3. A model LS-160 liquid nitrogen container
4. An Elektronik One-Eleven Single Pen Strip Chart Recorder (Honeywell International, Fort Washington, Pa.)

Ficoll-Hypaque will be used for the separation of lymphocytes from peripheral blood (5). A standard method of freezing live lymphocytes will be followed (6) and cell samples will be stored in a mechanical freezer at a constant -70°C .

RESULTS : Due to long procurement and delivery times, all of the components for the system were not received until recently. In addition the BF-4 rate controller was found after extensive testing to be defective and had to be returned to the Manufactures for replacement. We are presently awaiting the replacement rate controller. Because of the above we have no results to date.

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