The Macrophage Electrophoretic Mobility Test for Malignancy

M. MICHAEL LUBRAN, M.D., PH.D.

UCLA School of Medicine, Harbor General Hospital Campus,
Torrance, CA 90509

ABSTRACT

Lymphocytes obtained from the blood of patients suffering from malignant disease liberate, when incubated with a basic protein obtained from carcinomatous tissue or the brain, a substance (slowing factor) which reduces the electrophoretic mobility of guinea pig macrophages. Lymphocytes from normal subjects or patients with non malignant tumors do not liberate this slowing factor.

The macrophage electrophoretic mobility test (MEM test) for malignant disease was developed by Field and Caspary in 1970 as a result of some unexpected results obtained during the study of the reaction of lymphocytes to basic proteins extracted from human brain and sciatic nerve.9 These proteins, termed encephalitogenic factors or EF, which had been isolated and partially characterized by these and other investigators,3 cause allergic encephalomyelitis or neuritis when injected with Freund’s adjuvant into guinea pigs. Field and Caspary wished to discover whether or not blood lymphocytes obtained from patients with carcinomatous neuropathy or degenerative diseases of the nervous system were sensitized to EF. Sensitization was detected, as described in detail, by measuring the effect of the products of interaction of lymphocyte and EF on the electrophoretic mobility of guinea-pig peritoneal macrophages.8 As expected, blood lymphocytes obtained from normal subjects and patients with benign neoplasms were not sensitized; sensitized lymphocytes occurred in patients with carcinomatous neuropathy and degenerative diseases of the nervous system. The unexpected finding was the occurrence of sensitized lymphocytes in all subjects in the group of patients with malignant neoplasms not of the nervous system, irrespective of type or situation of the lesion. This finding, which has been confirmed by independent investigators,14,15 provides the basis for the MEM test.

Principle of the MEM Test

Sensitized lymphocytes, obtained from the blood of patients with malignant disease, when incubated with EF or certain other specific basic protein antigens produce a substance, probably a protein,2 which reduces the electrophoretic mobility of macrophages. It is believed that this substance, macrophage slowing factor or MSF, occurs on the plasma membrane of sensitized lymphocytes from which it is released by the antigen.6 MSF may be
identical with the migration inhibiting factor (MIF)\textsuperscript{12} produced by sensitized lymphocytes, which inhibits migration of macrophages. The electrophoretic mobility of the macrophages in the MEM test is measured in a Zeiss cytopherometer.

**Performance of the Test**

Antigen, patients' lymphocytes and guinea pig macrophages are required. Antigen may be EF prepared from human brain\textsuperscript{3,4} or basic protein obtained from human malignant tumors or from chronic lymphatic leukemic (cll) cells.\textsuperscript{4,7} Essentially, brain, tumor, or cll lymphocytes are homogenized and rendered lipid-free by prolonged extraction with a chloroform-methanol mixture, followed by extraction of the lipid-free residue with hydrochloric acid, pH < 3.5. Basic protein is precipitated from the extract with ammonium sulphate. Specificity is improved and convenience gained by the use of cll cells.\textsuperscript{7}

Patients' lymphocytes may be obtained from defibrinated venous blood by the modification by Hughes and Caspary\textsuperscript{12} of the method of Coulson and Chambers,\textsuperscript{5} or by the method of Pritchard et al.\textsuperscript{15}

Guinea-pig macrophages are obtained from the peritoneal exudate of uninfected Hartley albino guinea-pigs resulting from the intra-abdominal injection of sterile mineral oil. The lymphocytes in the macrophage suspension are inactivated by subjecting it before use to 100 to 200 rad from a $^{137}$Cs or $^{60}$Co source.

All measurements are made in TC 199, at pH 7.2. To $0.5 \times 10^6$ of the human test lymphocytes in 0.5 ml of TC 199 are added $10^7$ macrophages in 1.0 ml of TC 199 followed by 1.6 ml of TC 199. For the test mixture, 0.1 ml of antigen solution (one mg antigen per ml of TC 199) is added to the same quantities of macrophages and lymphocytes and finally 1.5 ml of TC 199. Both control and test mixtures are allowed to stand at room temperature ($20^\circ$ to $23^\circ$) for 90 minutes before examination. Pritchard et al.\textsuperscript{15} describe a slight variation of this procedure. In order to eliminate observer bias, tests are coded and randomized by another person before being examined by the observer. The mixtures are transferred to the cytopherometer, and macrophage mobilities measured.

The Zeiss cytopherometer\textsuperscript{*} is a combined horizontally-mounted microscope and vertical electrophoresis system. The electrophoretic chamber, which is thermostatically controlled, is connected to two non-polarizable electrodes. When a constant, direct current is passed between the electrodes, a stationary layer is formed in which no movement of fluid occurs. Cells moving in this layer from negative to positive electrode, are observed through the microscope, using bright-field, phase-contrast illumination. One eyepiece contains a reticule, the distance between the lines corresponding to 1.25 $\mu$ in the specimen plane. By concentrating on the forward edge of a cell, its migration rate in the electric field can be measured by timing its movement over the distance between several lines. The time should be about three seconds, measured with a stop watch to the nearest 0.1 second. The movement of the same cell in the reverse direction is timed after reversal of polarity of the electrodes, achieved by a switch. Macrophages are readily recognized by their mineral oil inclusions. Only those cells are timed which are sharply in focus in the stationary plane and which move without collision with other cells and without wandering out of focus.

Further, the forward and backward migration times must agree within 0.3 seconds. The transit times of 10 macrophages are measured, giving 20 readings from which the mean transit time is calculated. In practice, many more than 10 macrophages are observed, as about 40 percent

\* Carl Zeiss, Inc., New York, NY 10018.
of the readings are rejected because they do not obey the criteria. In any group of macrophages in the MEM test, there will be some that remain unaffected by MSF. These cells move faster than the affected cells and form a distinct and easily recognized subgroup. Their transit times are also rejected. The transit times of the remaining cells are very closely grouped about their mean. Percentage change in mobility is calculated as 100 \((T-C)/T\); the mean transit time of the macrophages in the test sample is \(T\) and in the control sample is \(C\). The mean transit time of macrophages incubated with antigen and lymphocytes from normal subjects varies little during many repeated observations.

### Results

Only two independent sets of investigators have, as yet, published results of the MEM test. Both report substantially the same results. Normal subjects give a slowing of 3.5 percent or less (negative), patients with malignant disease a slowing of 13 percent or more (positive). No overlap of the two classes has been observed. The MEM test is negative in patients with benign neoplasms and positive in patients, without malignancy, suffering from degenerative changes in the nervous system or old head injuries or minor strokes. These false positive results are eliminated if EF antigen is replaced by one derived from malignant growths. Positive results are obtained in some patients with sarcoidosis or systemic lupus erythematosus; the test is negative in patients with lymphatic leukemia. The MEM test, as described, is less sensitive in patients with advanced malignant disease due to the removal of sensitized lymphocytes by the tumor mass. The sensitivity of the test can be restored by increasing the number of lymphocytes used. The kinetics of the test have recently been studied.

No information is yet available about the results of the MEM test in patients with a wide variety of non-malignant diseases. However, it is not expected that there will be many false positives. Many tissues have been examined for basic protein (tumor antigen or CaBP). It has not been found in normal tissues or hypertrophic non-malignant tissues. CaBP is present in the lymphocytes of chronic lymphatic leukemia, but these lymphocytes do not react in the test as they cannot be stimulated by the cancer antigen to produce MSF. The experimental evidence suggests strongly that there is a common tumor antigen responsible for lymphocyte sensitization. The lymphocytes of chronic lymphatic leukemia provide a convenient source of CaBP. It has been found that the antigen develops rapidly in normal human embryonic tissue grown in vitro.

### Diagnostic Value of the Test

The MEM test presents technical problems which make it, at the moment, difficult to use in a non-specialized laboratory, although it is of great value as a research tool in the study of lymphocyte sensitivity. Once positive, the test remains positive, even though the tumor is completely removed. It is not, therefore, suitable for the diagnosis of metastatic or recurrent cancer. Further, the test detects the existence of cancer but not its type or location. Its major diagnostic value would thus appear to be for visible or readily detected lesions, including tumors of the skin, bronchi, breast, gastro-intestinal tract, uterus, prostate, eye, and brain. Used in conjunction with clinical data, the MEM test could be of great value in these cases. The false positives which occur using EF antigen are eliminated when tumor-derived antigen is employed. Only some cases of sarcoidosis and systemic lupus erythematosus have been reported to react positively with this anti-
gen. Even these false positives should be eliminated if all lymphocytes are used to provide antigen.

To make the test suitable for routine use, some simplification would be required. The preparation of the antigen, although tedious, is not difficult. Enough could be made at one time to suffice for a large number of tests. The separation of lymphocytes takes a few hours, but many specimens could be prepared at the same time. The production of the macrophages presents a problem. Animal house facilities and radioactivity equipment, not often available to routine laboratories, are necessary. Irradiation of the macrophages can be eliminated by a minor modification of the test, however, it is not then as discriminatory as in the unmodified version. If the test became popular, it is conceivable that some commercial supplier would be venturesome enough to provide both antigen and macrophages. The cytopherometer, although initially difficult to use, can be mastered and gives reproducible results in experienced hands. There is little doubt that the instrument could be simplified.

The results of the test in its present form are sufficiently encouraging for it to be evaluated in a wide variety of disease states, in addition to tumors. These disease states have not as yet been adequately tested. If the MEM test remains specific for malignancy, or has only a few false positives, it will prove a valuable addition to the slender battery of proven cancer tests.

References


