CELLULAR ELECTROPHORETIC TECHNIQUES SHOW PLASMA ABNORMALITIES IN MULTIPLE SCLEROSIS

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Analytical particle electrophoresis has been used in tests for the early diagnosis of multiple sclerosis (MS). The addition of linoleic acid (LA) to a suspension of fresh red blood cells (RBC) from MS patients in Medium 199 reduced their mean electrophoretic mobility (EPM). We found no significant change in the mobility of normal fresh RBC after the addition of LA unless they had undergone prior incubation in blood group compatible plasma from MS patients. When saline washed RBC from MS patients were incubated in normal plasma they then acquired normal electrophoretic properties in the fresh RBC test for MS.

While working toward the elucidation of the mechanism of this test we found that glutaraldehyde fixed RBC suspended in phosphate buffered saline (PBS), had an increased EPM after the addition of LA, with the mobility of RBC from MS patients still remaining lower than that of normal RBC. Polystyrene latex particles (PSL) were also used as carrier particles by incubating them in plasma from either MS patients or healthy subjects. The EPM of the plasma coated particles increased after the addition of LA, with the mobility of MS plasma coated PSL being less than those coated in normal plasma. This effect is similar to that found when RBC are used as carrier indicator particles. However, the results with PSL demonstrates that these electrophoretic phenomena originate from a plasma component abnormality (possibly a deficiency) in the blood of MS patients, rather than an intrinsic cell membrane defect. A group of MS patients in the early phases of the disease were given plasma infusions during acute exacerbations. Not only did they undergo neurological improvement, but their fresh RBC EPM test for MS went from positive into the normal range and remained there for 30 to 60 days after the infusion.

Memo:

THE APPLICATION OF CELL ELECTROPHORESIS TO RENAL TRANSPLANTATION

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Assessment of the immune status of renal transplant patients by in vitro techniques has proved of little use in clinical practice. We have developed an electrophoretic technique for assaying lymphokine production following lymphocyte-antigen reaction which requires four hours to perform.[1] This test has allowed assessment of the following parameters of the immune status of renal transplant patients.

a) The predictive value of the pretransplant reaction to donor cells. The measurement of the donor-recipient mixed lymphocyte reaction has proved to be a reliable prognostic index of graft survival in a 6 year study.

b) Plasma suppressive activity. The presence of a high level of PSA has been shown to be a good indicator of graft success.

c) Monitoring of acute rejection. Assessment of graft recipient lymphocyte reaction to both donor specific and organ specific antigens indicated impending acute rejection of the transplanted graft.

Conclusion. The use of this test before transplantation will greatly assist selection of the most suitable potential recipients for a given kidney and its use after transplantation will allow an early diagnosis of acute rejection.

1. Shenton B.K., et al. In, Cell Electrophoresis in Cancer and other Clinical Research. Ed. Preece A.W. and Light P. 1981, p.99.

Memo:

THE ROLE OF SURFACE NEGATIVE CHARGE IN PLATELET FUNCTION

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The role of surface negative charge in platelet function, especially in agglutination, was studied. Platelet electrophoretic mobility (EPM) as well as conductance in the suspension medium were measured with an automatic Laser Zee System 3000 (Pen Kem, U.S.A.). A good linear correlation was obtained between the whole platelet sialic acid amount and the EPM. Treatment of platelets with neuraminidase showed that the dose-dependent decreases in platelet EPM were paralleled by decreases in platelet bound sialic acid with concomitant increases in the sialic acid in the supernatants.

Ristocetin, an antibiotic known to agglutinate platelets in the presence of von Willebrand Factor (vWF), decreased platelet EPM dose-dependently without any change in the medium conductance in the absence of vWF.

Neuraminidase-treated platelets with EPM of about 50% that of controls retained about 100% of ristocetin-induced agglutination. Treatment of platelets with chymotrypsin resulted in the selective loss of platelet membrane glycoprotein-Ib shown by the PAS-stained SDS-polyacrylamide electrophoresis. The correlation curve between amounts of glycoprotein-Ib and the EPM suggested that the protein carried about 15% of whole platelet surface charge. The chymotrypsin-treated platelets with an EPM 85% that of controls lost almost all the agglutinability in the presence of vWF. The control and the enzyme-treated platelets decreased their EPM equally in the response to ristocetin alone. A small dose of vWF enhanced the decreases in the control platelet EPM induced by ristocetin but not in the chymotrypsin-treated platelets. When vWF had bound to glycoprotein-Ib in the presence of ristocetin, the local negative charge of the glycoprotein might be decreased. These results suggested that the neutralization of the surface charge on the site of glycoprotein-Ib plays an important role in ristocetin-induced agglutination.

Memo:

CELL ELECTROPHORETIC ANALYSIS OF POLYMORPHONUCLEAR CELLS IN COLLAGEN DISEASES

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Many reports have been described on electrophoretic changes of peripheral blood lymphocytes from patients with the various diseases, i.e. lympho-proliferative diseases. Electrophoretic analysis of polymorphonuclear cells was recently reported in rheumatoid arthritis patients (Brown, et al., 1981). As polymorphonuclear cells have functions such as chemotaxis and phagocytosis, it is important to investigate change of surface charge of polymorphonuclear cells.

Polymorphonuclear cells were isolated from peripheral blood. Analytical cell electrophoresis (manufactured by Sugiura Lab. Co., Tokyo) was carried out on samples containing 1×10^6 /ml in a cylindrical chamber. Subjects were studied on normal individuals and collagen disease patients.

Normal polymorphonuclear cells were electrophoretically distributed into two or three groups. Mean electrophoretic mobilities of polymorphonuclear cells from collagen disease patients (particularly, RA) were reduced. Electrophoretic change of each group was investigated. Function of polymorphonuclear cells from the patients with Behcet's diseases have been shown to be abnormal. Electrophoretic changes of polymorphonuclear cells was also studied in Behcet's diseases.

Memo:

ELECTROPHORETIC MOBILITY TEST FOR GYNECOLOGICAL MALIGNANCY

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Objectives; Lymphocytes from patients with malignant disease can be stimulated by encephalitogenic protein (E.P.), as a consequence of which a macrophage slowing factor (MSF) is released. This substance is absorbed by tanned sheep erythrocyte cells (TSRBC), and as a result the electrophoretic mobility becomes slow. The modification is named TEEM test, with which we tried to diagnose gynecological malignancy.

Material & Method; (1) E.P. was extracted by the method of Field & Caspary (1970). (2) Lymphocytes were isloated from patients with gynecological malignancy and those without malignancy (controls) by differential centrifugation. (3) TSRBC were prepared by the method of Stavitsky (1954). (4) Lymphocytes were incubated with 100μg E.P. for 60min. at 37°C. (5) The incubation mixture was then centrifuged and the supernatant in MSF was absorbed by TSRBC. (6) TEEM test was measured on Cytopherometers (Carl Zeiss). (7) The parcentage of slowing was culculated as follows : (Mean time of test mixture)-(Mean time of control)/(Mean time of control)x100 (8) The slowing was estimated as follows : >7%=negative ≦7%=positive results.

Results; The positive % slowing occurs in 88.2% of malignancy patients who have verified histological carcinoma. Non-malignant patients show 8.7%, normal subjects show 2.9% positive slowing. The products of MSF was not only lymphocytes but also macrophages was needed. The material of MSF resumble as MIF be suspected.

Memo: