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USE OF MONOCLONAL ANTIBODIES AGAINST MYOSIN LIGHT CHAINS 1 TO DETECT THE CORRESPONDING ANTIGEN IN BLOOD OF PATIENTS WITH MYOCARDIAL INFARCTION

E. V. Pulyaeva, A. L. Konorova, L. I. Kovalev, A. Yu. Volgin, S. S. Shishkin, and L.A. Pevnitskii

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The human myocardium, an example of highly differentiated tissue, is characterized by a very specific set of contractile proteins. It has been shown, in particular, that expression of a special active gene takes place in heart muscle, and this is accompanied by the corresponding synthesis of a tissue-specific form of actin [7]. By contrast with this, the tissue-specific form of α-tropomyosin is formed, not through expression of a special gene, but by means of alternative splicing of pre-mRNA of the gene which determines synthesis of the α-tropomyosins of skeletal and smooth muscles [6]. Myosin light chains 1 (MLC1) also are tissue-specific, and even the existence of different isoforms of MLC1 has been demonstrated in the myocardium of the ventricles and atria [3].

Previously the writers described obtaining a hybridoma clone, producing antibodies which "recognize" ventricular MLC1 of human heart muscle, but do not react with myosin light chains from other tissues [2]. Degenerative changes in the myocardium (especially infarction) may be accompanied by the appearance, not only of cytosolic enzymes of heart muscle, but also of certain contractile proteins, in the blood plasma [5].

In this investigation an attempt was made to use monoclonal antibodies obtained previously to look for the corresponding antigen in the blood plasma of patients with myocardial infarction, for it might become a highly specific marker of myocardial dystrophy.

EXPERIMENTAL METHOD

The test object consisted of samples of venous blood from four patients with myocardial infarction, obtained in the active phase of the disease. The diagnosis in all cases was based on EEG data and determination of transaminase activity and other biochemical tests. Blood samples from four healthy individuals served as the control. The cells were separated from the plasma by centrifugation. Preparations of plasma were used for enzyme immunoassay (EIA). Monoclonal antibodies produced by clone MLC-1c were obtained as described previously [2]. Polyclonal antibodies against MLC1 of the human ventricular myocardium were prepared from serum of rabbits immunized with the corresponding antigen by the method in [4]. The content and specificity of polyclonal antibodies were verified by carrying out immunoblotting of the zone of myosin light chains by two-dimensional electrophoresis of extracts of human heart muscle [2]. The antibody titer was determined in

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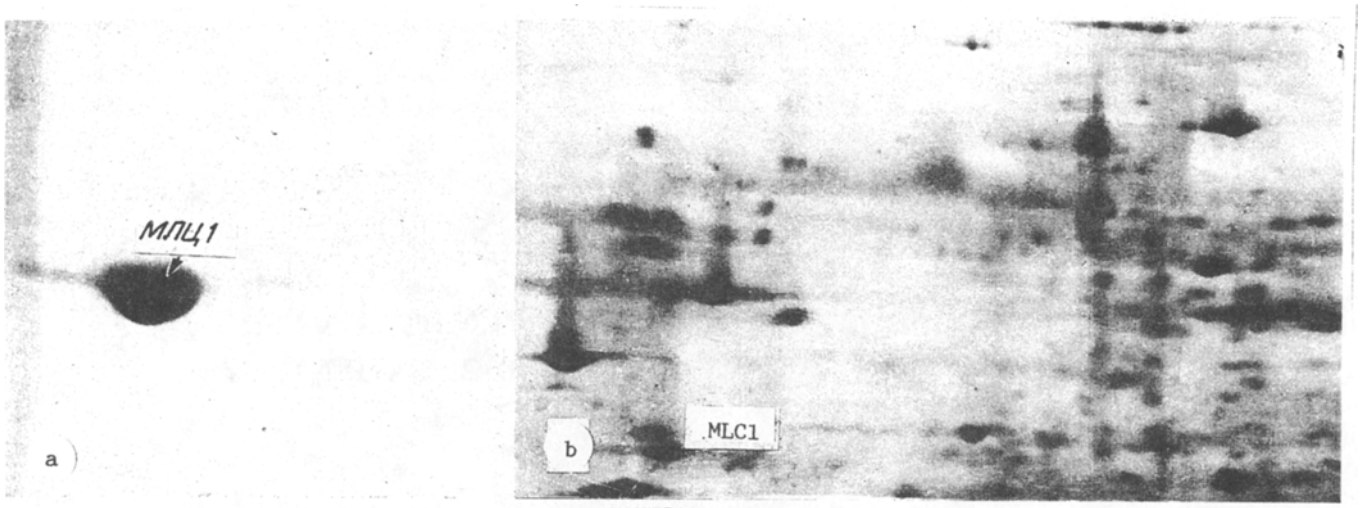

Fig. 1. Two-dimensional electrophoresis of purified MLC1 preparation (a) and total homogenate of left ventricle of human heart (b).

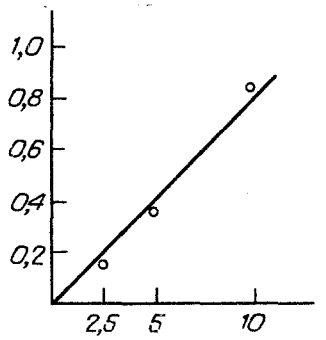

Fig. 2

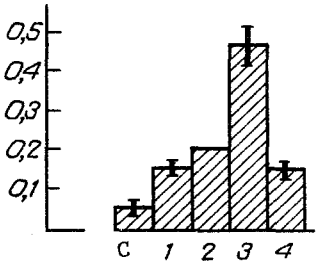

Fig. 3

Fig. 2. Typical calibration curve for EIA. Abscissa, concentration of MLC1 (in ng/ml); ordinate, OD.

Fig. 3. Antigen concentration in blood plasma of healthy individuals (C) and patients with myocardial infarction (1-4). Vertical axis — OD.

EIA by the serial double dilutions method. The preparation of antigen for immunization and for construction of a calibration curve for EIA was obtained from autopsy material of human heart muscle in two stages: initially, as described in [8], partially purified myosin was isolated, and this was later fractionated by two-dimensional electrophoresis by O'Farrell's method. The MLC1 fraction was detected, eluted, and obtained as described previously [2].

The antigen was tested by EIA in the "sandwich" version. Initially monoclonal antibodies produced by clone MLC-1c were immobilized on the surface of 96-well planchets ("Costar," USA). Into each well was introduced 100 μl of 10 mM carbonate buffer, pH 9.5, containing antibodies (40 μg protein in 1 ml), and the planchets were incubated for 3 h at 37°C. The solution was then poured off and the wells washed 3 times with rinsing buffer (TBS, 0.05% Tween-80), after which 200 μl of TDS containing 2% bovine serum albumin (BSA) was added to each well, and the planchets were allowed to stand overnight at 4°C. The wells were then washed as described above and samples to be tested for their antigen content were introduced into them. A sample with a volume of 100 μl was added to each well. The samples were made up in TBS-buffer containing 0.05% Tween-80 and 0.6% BSA. During analysis of human plasma, no albumin was added to the above solution. The samples were incubated for 3 h at 37°C. The incubation solution was then removed and 100 μl of TBS-buffer, containing 0.05% Tween-80 and 0.6% BSA, and also rabbit blood serum containing polyclonal antibodies in a

dilution of 1:1000 were introduced into the wells. Incubation continued for a further 3 h at 37°C. The wells were then rinsed and conjugate of goat antibodies against rabbit immunoglobulins with horseradish peroxidase was added to them. The planchets were then incubated in TBS buffer containing 0.05% Tween-80 and 10% human plasma. This last component was added to abolish nonspecific binding of the conjugate with the first antibodies. Immune complexes were revealed by incubation in 0.05 M phosphate-citrate buffer, pH 5.0, containing 0.04% orthophenylenediamine and 0.005% hydrogen peroxide for 30 min at room temperature in darkness. The reaction was stopped by addition of concentrated sulfuric acid to 8 N, and the optical density (OD) was measured on a "Multiscan" instrument (Flow Laboratories, England). Values of OD in wells to which the antigen solution was not added served as the background. Three determinations of OD were made on each sample, and the results were analyzed by calculation of the arithmetic mean.

EXPERIMENTAL RESULTS

Electrophoresis of the isolated MLC1 preparation, used as antigen to immunize the animals and to plot the calibration curve for EIA, is shown in Fig. 1a and compared with the result of electrophoresis of the original total homogenate of the left ventricle of the human heart (Fig. 1b).

The existing highly specific monoclonal and polyclonal antibodies served as the basis for development of a system enabling the MLC1 antigen to be found with a high degree of sensitivity in the test samples. The use of the sandwich version of EIA in this case enabled the antigen to be concentrated on the first layer of antibodies. This made its results more reproducible, and the version itself is the most sensitive of all forms of EIA [1].

The first step in the development of the system was construction of a calibration curve showing OD as a function of antigen concentration in the sample. Serial double dilutions of MLC1 in TBS buffer were used. It will be clear from Fig. 1 that dependence of the values of OD on antigen concentration in this system of analysis is a linear function, and the angle of slope of the straight line is close to 45°, so that double dilutions of antigen can be reliably distinguished. The linear nature of the response was observed over a range of protein concentrations in the sample for analysis of between 0.00025 and 0.001 μg, or 2.5-10 mg MLC1 in 1 ml of sample. Scatter, estimated from three parallel measurements on the same specimen, varied from 0.05 to 0.1% of OD.

The use of this system to assess the concentration of the given antigen in blood plasma from four healthy individuals showed that under these circumstances the values of OD did not differ from the background values.

A diagram enabling the results of EIA for determination of antigen concentration in blood plasma from healthy individuals and four patients with myocardial infarction to be compared in relative units is given in Fig. 3. Antigen in different titers could be found in the blood plasma of all the patients by the use of this analytical system.

Thus since the system developed for finding the antigen can be regarded as highly specific, the antigen tested is evidently myosin light chain 1 from the ventricles of the human heart. In conclusion, it may be noted that the suggested system will probably be able to detect specific dystrophic changes in the human myocardium and, in particular, it will distinguish between lesions in the ventricles and in the atria.

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