DEVELOPMENT AND CLINICAL TRIALS OF A MICROMODIFICATION OF THE HUMAN BLOOD LEUKOCYTE MIGRATION INHIBITION TEST

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A new modification of the human blood leukocyte migration inhibition test is suggested. The results of clinical trials enable it to be recommended for monitoring the dynamics of cellular immunity and the level of humoral factors in patients with various immunopathological states. By means of the suggested modification and using small amounts of blood, 10 to 15 parallel tests can be carried out simultaneously.

KEY WORDS: *inhibition of leukocyte migration.*

In recent years the leukocyte migration inhibition test (LMIT) has been used very extensively in clinical and experimental practice for the study of cellular immunity in vitro. The method is highly sensitive and specific and its results correlate well with those of other tests characterizing the state of hypersensitivity of delayed type. The LMIT was first used clinically [2] to study ulcerative colitis, but later in autoimmune diseases, malignant neoplasms, and after transplantation of organs. However, the wider use of this method in clinical practice is prevented by the need to use large quantities of blood, which makes repeated tests on patients at intervals difficult. In the last 2 years micromodifications of the LMIT have appeared. They include a modification in which a mixture of guinea pig macrophages and 5% of human lymphocytes is used [3]. This method gives good results, but the need to use animal macrophages makes it unsuitable for clinical application. In another modification [1] whole blood and specially made capillary tubes (pyloscopic tubes of Perfil'ev and Gabe) were used. This method is sufficiently simple and requires only small volumes of blood for the tests, but it does have limitations because it takes no account of humoral factors which may affect the cellular response.

This paper describes a new suggested micromodification of LMIT. The results of trials of this modification for the investigation of cellular immunity and humoral factors in patients also are given.

Preparation of the Cell Suspension. Heparinized blood (4-5 ml) was incubated for 15-30 min to sediment the red cells, after which the two blood fractions obtained were centrifuged to remove the plasma. The top half of the cells, contaminated with leukocytes, was withdrawn from the tube containing erythrocytes and transferred to a clean tube. Two fractions of blood (pure leukocytes and erythrocytes with a few leukocytes) thus obtained were washed 3 times with medium No. 199. The residue of leukocytes was treated with 0.2-0.3 ml medium No. 199 and thoroughly mixed, after which the erythrocytes were transferred into the same tube. The mixture of cells was again stirred and divided into two parts. Healthy serum from a group IV blood donor was added to one part of the cell suspension, autologous serum to the other in the ratio of 2:1.

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TABLE 1. LMIT on Patients with Malignant Bone Tumors

Patient	MI with sera		Effect of autologous serum
	homologous	autologous	
F.	0,5	0,8	Blocking
L.	0,6	1,2	"
R.	0,4	0,4	Absent
M.	0,1	0,6	Blocking
M.	0,3	0,9	"
V.	0,9	0,6	Intensifying
A.	0,6	0,9	Blocking
U.	0,6	1,1	"

TABLE 2. LMIT on Patients with Clinically Demonstrable Hypersensitivity to Therapeutic Substances

Substance	Migration index of patients' cells	
	T.	G.
Sulfoditoxin	—	1,00
Analgin	0,80	0,10
Amidopyrin	—	0,60
Garamycin	—	0,64
Dalacen	—	2,00
Lincomycin	—	0,90
Ceparin	0,79	1,00
Rondomycin	—	0,25
Nevigramon	— —	0,70
Erythromycin	— —	0,90
Monomycin	—	0,94
Novobiocin	—	0,59
Gentamycin	0,65	—
Thiopental sodium	—	0,20
Droperidol	—	0,53
Ketalar	—	0,85
Promedol	0,79	—

Antigens. In the corresponding investigations a saline extract of osteogenic sarcoma previously homogenized by means of an ultrasonic generator, and a standard preparation of γ-globulin (prepared from human blood and used for measles prophylaxis), previously incubated at 56°C for 30 min, were used as antigens. The final concentration of antigens was 20-100 μg/ml. The sensitivity of the patients' cells to various therapeutic substances in a final concentration of 20 to 500 μg/ml also was studied.

Setting up the Test. To 0.2 ml of the cell suspension, placed on a slide, 0.05 ml of antigen was added in the appropriate dilution and the mixture was carefully stirred with a glass rod. The plate formed from five capillary tubes was filled with the mixture to about 2/3 of its length (Perfil'ev–Gabe capillary tubes, made to É. P. Troshanov's modification by the Group for Capillary Microscopy, Institute of Limnology, Academy of Sciences of the USSR). The ends of the plate were coated with Mendeleev's paste. Meanwhile another plate of capillary tubes was filled with the same cells, but without antigen. The capillary tubes were centrifuged for 5-7 min at 1000 rpm and left to stand for 24 h at 37°C in the horizontal position. In all cases, healthy human cells were tested at the same time as patients' cells with the same antigen. All tests were repeated 2-3 times. The test was thus evaluated on the basis of 10-15 parallel determinations.

Reading the Reaction. The reaction was read under the microscope with a magnification of 16 times. The length of the path covered by the blood leukocytes from the boundary with the erythrocytes was measured with a graduated ocular, after which the migration index (MI) was calculated as the ratio between the length of the path of the leukocytes in the presence of antigen (L_{ag}) and the length of the spontaneous path without antigen (L_{sp}):

$$MI = \frac{L_{ag}}{L_{sp}} .$$

Each antigen was investigated preliminarily with the blood cells of 13-15 healthy persons. MI for healthy human leukocytes was 1 ± 0.2.

The reaction was carried out under sterile conditions in siliconized glassware.

Groups of Patients Investigated. For trials of the method the following patients were tested: nine patients with rheumatoid arthritis (seven children and two adults), 14 patients with malignant tumors of bone in various situations, and also two patients (scoliosis and rheumatoid arthritis) with clinically manifested hypersensitivity to various therapeutic substances.

The LMIT on the patients with rheumatoid arthritis was carried out with γ-globulin, which is also used in serological tests for determination of rheumatoid factor. Rheumatoid factor is very rarely detected in children with rheumatoid arthritis, so that the diagnosis of this disease in childhood is difficult. A positive LMIT with γ-globulin was obtained in four of the seven children tested and in two adult patients

with rheumatoid arthritis. MI in the various patients varied from 0.8 to 0 (mean MI = 0.49 ± 0.05). It will be noted that inhibition of migration in the presence of γ-globulin was observed in "seronegative" children with the disease, a matter of possible practical importance.

Addition of patients' serum to the medium in no case altered the results of the test. In control tests on 13 clinically healthy persons γ-globulin did not cause inhibition of the migration (MI = 0.96 ± 0.07).

The next step was to investigate 14 patients with malignant bone tumors in various situations. In seven of them some degree of inhibition of migration in the presence of antigens of a tissue extract of osteogenic sarcoma was observed on admission to hospital. In eight patients the effect of autologous serum on the value of LMIT was studied. The results of the LMIT with homologous and autologous sera are given in Table 1.

It will be clear from Table 1 that the serum of six patients had a blocking action and completely abolished or reduced the effect of inhibition of cell migration by antigen. The serum of patient V. intensified the LMIT, but that of patient R. had no effect. It is interesting to note that in patient V., whose serum intensified the LMIT, the clinical course of the osteogenic sarcoma was torpid, unaccompanied by metastasization, and giving no recurrence for a long period of time after the operation.

In control tests on 14 healthy subjects the tumor antigen did not cause inhibition of leukocyte migration.

Patients with hypersensitivity to various therapeutic substances are not infrequently found in clinical practice, and administration of these substances may give rise to serious clinical complications. It was decided to investigate the sensitivity of the cells of two such patients and to compare the results of the LMIT with the clinical manifestations of hypersensitivity. The results of these investigations are given in Table 2.

As Table 2 shows, a particularly strong reaction to analgin, amidopyrin, rondomycin, novobiocin, thiopental sodium, and droperidol was observed in patient G. The results of the LMIT correlated with the clinical picture. For instance, intramuscular injection of analgin into this patient gave rise to an anaphylactoid reaction. Injection of preparations which did not inhibit cell migration in vitro into the patients likewise did not cause clinical symptoms of allergy. In two healthy subjects used as controls, no reaction was observed to these substances.

It can accordingly be concluded from these results that the proposed micromethod of the LMIT enables cellular immunity and humoral factors to be studied, with the use of only small quantities of blood, in patients with various immunopathological states. With strict observance of the conditions, the method ensures good reproducibility of the results, and if the reaction is read on the basis of 10-15 parallel tests, the results are reliable.

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