A MICROMETHOD OF CULTIVATING HUMAN BLOOD CELLS

I. I. Kopelyan and M. P. Grigor'eva

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Micromodifications of the blast-transformation reaction of human peripheral blood lymphocytes to phytohemagglutinin and in a mixed culture of lymphocytes are described. The effect of different conditions of cultivation on the survival and reactivity of the lymphocytes in culture is demonstrated. The results obtained by micro- and macromodifications of the reaction are compared.

Cultures of lymphocytes are being increasingly used both to study the mechanisms of transplantation immunity and in clinical and experimental practice. The first attempts to use a micromethod of cultivating blood cells were concerned with the cultivation of whole blood for investigation of both specific and nonspecific stimulators of blastogenesis [2-5]. Later a micromethod was used for the selection of a donor for organ homografting [1].

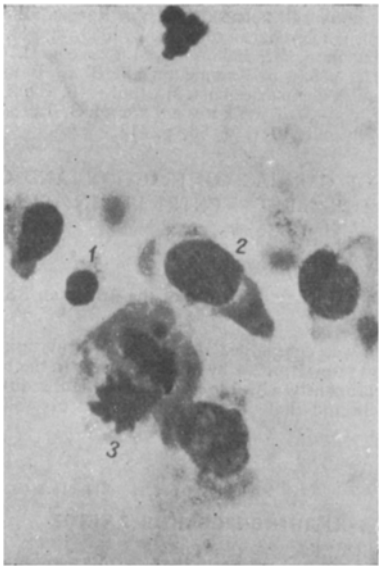

Fig. 1. Blast-transformation reaction in a monoculture of cells in the presence of PHA, 630×: 1) small lymphocyte; 2) blast cell; 3) mitosis.

The object of the present investigation was to develop two technically simple and highly reproducible micromodifications of the blast-transformation reaction (BTR): with phytohemagglutinin (PHA) and in a mixed culture of lymphocytes (MCL), which would require the minimal volume of blood for its performance, an important matter where prolonged dynamic testing of the patients' immunological reactivity is required.

MACROMODIFICATION OF THE REACTION WITH PHA

Whole blood taken from the subject's finger was used. The finger tip was first painted with iodine and alcohol and then punctured with a scarifier. Blood (0.1-0.2 ml) was taken with a micropipet, wetted with heparin solution, made up from dried heparin (Spofa, Czechoslovakia) in the proportion of 5000 units to 1 ml distilled water. Next, 0.1 ml blood was mixed with 5 ml medium No. 199 containing 20% bovine serum. Penicillin (200 units) and streptomycin (100 units) were added to 1 ml nutrient medium. The cells were cultivated in 20-ml tubes made from neutral glass for 3 days at 37°C. The tubes were placed in an incubator.

After cultivation for 3 days 4 ml of the nutrient medium was drawn off with a Pasteur pipet. The residue of cells was carefully pipetted into the residual medium so as to destroy the conglomerates of blast cells formed during cultivation with PHA. The first

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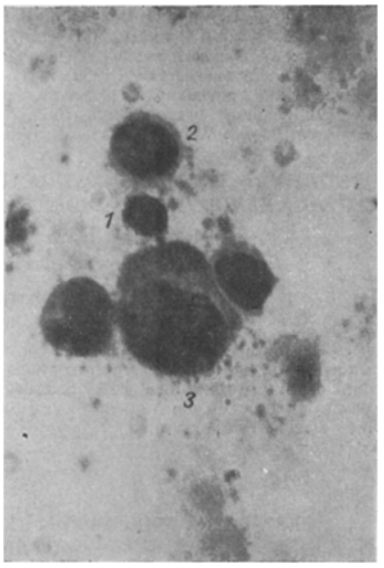

Fig. 2. Blast-transformation reaction in mixed culture of homologous lymphocytes, 630×: 1) small lymphocyte; 2) transition form of cell (from small lymphocyte into blast cell); 3) blast cell.

TABLE 1. Reproducibility of Reaction of Lymphocytes of Healthy Persons to PHA when Performed on the Same Day and on Different Days

Time of performing test	Mean percentage	Reproducibility (in %)
On same day	72,0—1,2	80
On different day	72,3—1,6	83

suspension was transferred into centrifuge tubes and centrifuged for 10 min at 90 g. The supernatant was carefully removed with a Pasteur pipet, the cells were carefully resuspended in the remaining drop of medium, and 8–10 ml of fixing solution was applied. The fixing solution was a mixture of three parts ethyl alcohol and one part glacial acetic acid. Incubation of the cells in the fixing solution for 10 min caused fixation of the white cells and lysis of the red cells. After fixation the cell suspension was centrifuged for 10 min at 90 g, the fixing fluid was removed, and the cells resuspended in a drop of fixing solution were transferred to a dry slide. After drying, the cells were stained with azure-eosin and the results read morphologically (the percentage of transformed lymphocytes per 200-300 cells was determined). The percentage of transformed cells included typical blast cells, transition forms, and also cells in state of mitotic division (Fig. 1). The results were assessed on the basis of not less than two parallel tests.

MICROMODIFICATION WITH MCL

For the mixed culture 0.8–1.5 ml blood was taken with a Pasteur pipet, transferred to precipitation tube, and incubated at 37°C for 5–15 min to sediment the red cells (to one-third of the volume of blood taken). Two-thirds of the supernatant plasma was then drawn off, poured into centrifuge tubes, and centrifuged for 10 min at 90 g. The residue of cells was washed once with medium No. 199. The cells after washing were mixed with the same medium containing 20% homologous group AB serum, 200 units penicillin, and 100 units streptomycin to 1 ml. After the cells had been counted in a Goryaev's chamber their concentration was adjusted to 1 million cells/ml. Seeding was carried out in 10-ml tubes made from neutral glass at the rate of 200,000 lymphocytes from each donor. The tubes were incubated at 37°C for 5 or 7 days depending on the object of the investigation. When the culture was removed the cells were taken out with a Pasteur pipet, blowing air through it to break up the possible conglomerates of cells arising during cultivation. A drop containing cells was placed on a slide to make into a film. After drying the films were fixed for 5–7 min in absolute methanol and stained with azure-eosin. The results were read as described above (Fig. 2).

RESULTS OBTAINED BY THE MICROMETHODS

When a standard dose of PHA (Wellcome), namely 0.1 ml in a dilution of 1 :10, was used the mean percentage of the BTR in healthy adults was 71 ± 4.2.

A special investigation showed the intensity of the reaction was independent of the absolute and relative numbers of lymphocytes in the subject's blood and showed high reproducibility both when carried out on the same day and on different days (Table 1).

The reproducibility of the reaction was determined from the results of not less than ten experiments by calculating the arithmetic mean (X) and the standard deviation. The percentage of all values between the confidence limits relative to the number of experiments (p = 95%) was determined.

The results of the MCL reaction were affected by the number of blood cells in the culture of lymphocytes. In these tests the ratio between the cell forms in the culture (initial figures on seeding) was as

TABLE 2. Reproducibility of Reaction of Lymphocytes of Healthy Persons in Mixed Cultures when Performed on the Same Day and on Different Days (on 5th day of cultivation)

Time of performing test	Mean intensity of reaction (in %)	Reproducibility (in %)
On same day	2,7±0,6	80
On different days	3,5–0,6	80

TABLE 3. Results of Comparative Investigations Using Macro- and Micromodifications of the Blast-Transformation Reaction

Blood donors	BTR (in %) to PHA		Blood donors	BTR (in %) in MCL (7 days)	
	macro-method	micro-method		macro-method	micro-method
1	70	72	1+2	9	8
2	69,5	70	1+3	23	20

follows (for 100% lymphocytes): granulocytes 15–50%, monocytes 2–2.5%, erythrocytes 200–300%, platelets 200–300%. Variation in the number of neutrophils (20–50%) did not affect the intensity of the MCL reaction (the intensity was 6 and 5.5%, respectively). The mean intensity of the reaction in the MCL on the 7th day was 8.8 ± 2.1%. The maximal intensity of the reaction observed during the investigation of mixed cultures was 20%.

This micromodification also showed high reproducibility of the reaction when performed both on the same day and on different days (Table 2).

In the study of the effect of different conditions of cultivation on the magnitude of the BTR and MCL, it was discovered that survival and reactivity of the lymphocytes were appreciably changed if CO_2 was blown through, but supersaturation with CO_2 may cause death of the cells in the culture. Partial or complete change of nutrient medium 2–3 days after the beginning of cultivation reduced the reactivity of the lymphocytes by 33–50%. Single daily or constant agitation of the tubes (or mixing of the cells) during cultivation appreciably reduced the survival rate of the cells and almost halved their reactivity. The best tubes for cultivation are those made of neutral glass with a round bottom.

Comparison of the results obtained by the macro- and micromodifications of the reaction to PHA and in the MCL gave consistent results (Table 3).

Small volumes of blood, a simple technique of performance and removal of the culture, and the highly reproducible results thus allow the micromodifications of the BTR induced by PHA or by allergeneic cells as described above to be recommended for the dynamic study of the immunological reactivity of patients.

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