CULTIVATION OF HUMAN PERIPHERAL BLOOD LEUKOCYTES IN AGAR GEL

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The use of a modified method of cloning hematopoietic cells in semisolid nutrient media showed that among human peripheral blood leukocytes there are committed precursor cells of the granulocytic series (CFU_C). In healthy adults and children (aged from 4 days to 10 years) the number of these precursors is 0.05-6.38 and 0.2- 2.9/10^5 nucleated cells respectively. The number of CFU_C in the blood of patients with infectious mononucleosis is within normal limits (0.5-14/10^5 nucleated cells). In acute leukemia very low colony-forming activity of the nucleated blood cells is found (0-0.3/10^5 cells).

KEY WORDS: cloning method; colony-forming cells; infectious mononucleosis.

By culturing hematopoietic cells in semisolid agar it is possible to determine the committed precursor cells of the granulocytes [1, 2]. In some animals (mice, rats) colony formation has been shown to depend on the presence of exogenous colony-stimulating factor (CSF), contained in the serum and urine of man and mice and also in various tissue extracts and culture media of hematopoietic cells, kidneys, lungs, or tissues of the whole embryo [3-6]. In other species and, in particular, in man, autonomous growth is observed in the absence of exogenous CSF in high cell concentrations [7]. Colony-forming cells (CFU_C) are found in man not only in the bone marrow but also in the peripheral blood [8, 9].

The writers have attempted to culture peripheral blood leukocytes from healthy persons and patients with blood diseases, using a closed system of culture in Leighton's tubes, as previously adopted for mouse bone marrow culture [10].

EXPERIMENTAL METHOD

Blood from 34 men and women of different ages was used.

McCoy's single medium for agar cultures (medium 1) was prepared as follows: McCoy's medium 5A 500 ml, 5% sodium bicarbonate solution 5.6 ml, MEM vitamins 100 × solution 2.5 ml, MEM essential amino acids 10 × solution 2.5 ml, MEM nonessential amino acids 50 × solution 5 ml, 2.2% sodium pyruvate 6.25 ml. This mixture was kept for several months at 4°C.

Modified McCoy's medium (medium 2) was prepared immediately before use by mixing the following ingredients: medium 1 83.5 ml, embryonic calf serum 15 ml, L-glutamine (200 mM) 0.4 ml, L-asparagine (20 mg/ml) 0.16 ml, penicillin and streptomycin, 20,000 units/ml of each, 0.25 ml.

The nutrient agar medium for blood culture (medium 3) was prepared immediately before the experiments by mixing nine parts of medium 2 and 1 part of a 3.3% aqueous solution of Bactoagar, and was kept at 4°C. The pH of the media was 7.2. The 3.3% solution of Bactoagar was made up in triple distilled water immediately before use, sterilized by boiling, and kept at 60°C.

The blood was taken into sterile tubes containing heparin solution (32 units/ml blood). The plasma was drawn off 1-2 h after sedimentation of the erythrocytes and the number of nucleated cells was counted.

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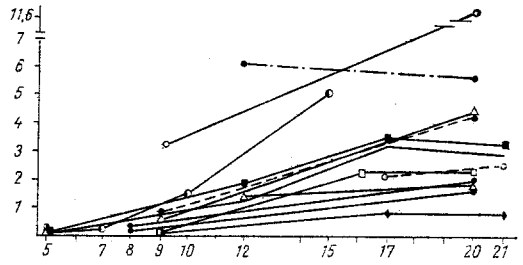

Fig. 1

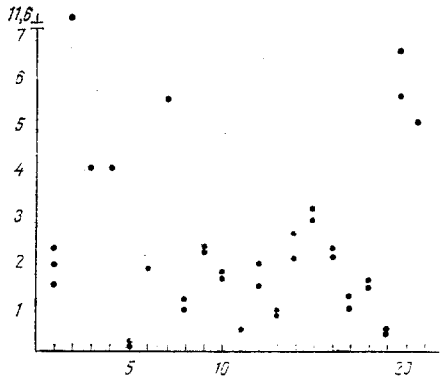

Fig. 2

Fig. 1. Number of colonies as a function of cultivation time. Circles, squares, and triangles represent different experiments. Abscissa, cultivation time (in days); ordinate, number of colonies per 10^5 nucleated cells.

Fig. 2. Number of CFU_C of human peripheral blood in different experiments. Abscissa, No. of experiments; ordinate, number of colonies per 10^5 nucleated cells.

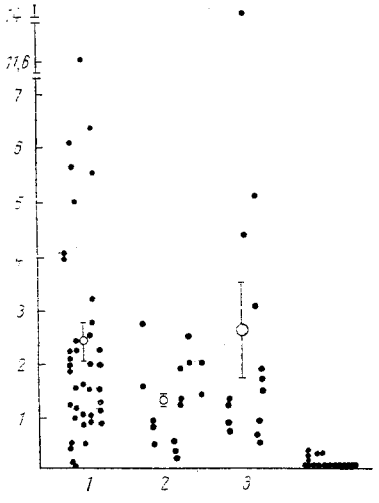

Fig. 3. Number of CFU_C from normal and pathological human peripheral blood. Abscissa, experimental groups: 1) healthy adults, 2) healthy children aged from 4 days to 10 years, 3) children with infectious mononucleosis aged from 3 to 5 years, 4) patients with acute leukemia; ordinate, number of colonies per 10^5 nucleated cells.

Leukocytes were added at the rate of 10^6 nucleated cells to 1 ml agar medium, carefully pipeted, and distributed among Leighton's tubes in a volume of 2 ml. Twenty minutes after gelatinization of the agar at room temperature the tubes were aerated for 10 sec with a mixture of air (90%) and CO_2 (10%). The tubes were sealed with airtight rubber stoppers and incubated at 37°C. Colonies were counted 10-20 days later under an inverted microscope with a magnification of 25-40. For cytological investigations the colonies were removed from the agar with a fine Pasteur pipet and applied to a slide. The colonies were stained with 0.4% orcein solution in 60% acetic acid, covered with a cover slip, and the cells were classified under a magnification of 400-1000. The colonies appeared on the sixth to tenth day and contained from 5 to 50 cells of the granulocytic series. During growth the colonies increased in size and became spherical in shape. Parallel with the increase in size of the individual colonies in the cultures, new colonies were formed right up to the 17th day of cultivation (Fig. 1). By the 18th-20th day the colonies attained their maximum development and each contained 200-800 cells.

In man, just as in mice, three types of colonies of hematopoietic cells can be distinguished. Colonies of the first type are compact, clearly outlined, spherical collections of cells, mainly cells of the granulocytic series from myeloblasts to mature granulocytes, with numerous mitoses but hardly any macrophages. Colonies of the second type have a dense, compact nucleus, containing cells of the granulocytic series at different stages of differentiation, and a halo of macrophages. The third type of colony is a loose spherical collection of mononuclear cells without a central nucleus.

EXPERIMENTAL RESULTS

The study of the colony-forming activity of the blood cells of 21 healthy persons aged from 18 to 45 years showed that the number of colonies after explantation of $2 \cdot 10^6$ nucleated cells per tube varied considerably in different subjects: The number of CFU_C varied from 0.05 to 6.38 per 10^5 nucleated cells (Fig. 2) and in one case the number of CFU_C reached $11.6/10^5$ nucleated cells.

The number of CFU_C in the peripheral blood of five healthy children aged from 4 days to 10 years varied from 0.2 to $2.9/10^5$ nucleated cells (Fig. 3), i.e., it was virtually indistinguishable from the number in the adults.

Investigation of blood from five children aged 3-5 years with infectious mononucleosis showed that the number of CFU_C was within normal limits (Fig. 3). Very low colony-forming activity was found in 11 patients with acute leukemia. In six cases cultures of leukemic cells gave no colonies, and in five other cases the number of CFU_C varied from 0.03 to $0.3/10^5$ nucleated cells (Fig. 3).

The system for culture of hematopoietic cells in agar described above thus enables precursor cells of the granulocytic series to be determined in human blood without the use of special incubators with controlled humidity and gas composition. Determination of CFU_C in the peripheral blood does not require the presence of CSF, evidently because the blood contains monocytes which produce CSF [7, 11, 12].

It is difficult to say what is the reason for the large variations (by 100 ×) in the number of CFU_C in healthy persons. They could be based on differences in the intensity of leukopoiesis in different subjects, differences in the time of day, differences in the activity of the cells producing CSF, and so on. The problem requires special study.

In infectious mononucleosis the number of CFU_C was unchanged, in good agreement with the presumed localization of the lesion in this disease in lymphopoietic but not in the granulocytopoietic cells.

The sharp decrease in the number of CFU_C in acute leukemia has been observed previously in the bone marrow [13]. It was accompanied by a correspondingly low level of CFU_C in the blood. It has been suggested that in acute leukemia the lesion is often localized at the CFU_C level, where ability to form colonies is lost [14]. It has also been suggested that in acute leukemia there are no cells producing CSF in the blood. This problem will be studied by the writers with the use of exogenous CSF.

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