PRODUCTION OF A MUTANT (HPRT$^-$) CELL LINE ON THE BASIS OF HUMAN ERYTHROMYELOLEUKEMIA K-562

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Human and animal cells differ in their ability to multiply in culture *in vitro*. This ability depends on the conditions of feeding, temperature, and many other factors. In most cases, it has been found convenient to select mutant cells on the basis of easily determinable features. A clone of mutant cells defective for a particular enzyme can be obtained by treating the normal population with a cytostatic, which is incorporated into nucleic acids with the aid of this enzyme. The first observations in this direction were made by Littlefield [7], who developed a general method of selection of mutants on the basis of their resistance to 8-azaguanine (8AG) and bromodeoxyuridine. On the addition of 8AG to the culture medium, it is incorporated with the aid of the enzyme hypoxanthine phosphoribosyltransferase (HPRT) into nucleic acids and kills cells which possess this enzyme. Mutant cells, without this enzyme, do not incorporate 8AG and thus survive.

Many mutant cell lines obtained from myelomas and leukemias of animals [1-3, 5, 6, 8, 9] and two mutant lines based on human myelomas, used for hybridization with antibody-producing lymphocytes, have been described in the literature. The first was obtained from myeloma U-266 and mutants were selected with the aid of 8AG [12]. The second line also was obtained from a myeloma and mutants were selected with the aid of 6-thioguanine [13].

The object of the present investigation was to select mutant cells with the aid of 8AG and to obtain a mutant line from a culture of K-562 cells maintained *in vitro* in the writer's laboratory for 2.5 years.

EXPERIMENTAL METHOD

A culture of human erythromyeloleukemia K-562, adapted in the writer's laboratory to medium 199 in Hanks' solution with the addition of 10% fetal calf serum (from Flow Laboratories, England, and the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow) and 20 μM glutamine (from Serva, West Germany), was transferred for selection to medium RPMI-1640 (Flow Laboratories, England; Gibco, USA) with 10% fetal calf serum and 20 μM glutamine, to which 8AG was added in a concentration of 20 μg/ml. During culture of the original K-562 cells, and later of the mutant cells, the antibiotic monomycin was added to the medium in a concentration of 100 U/ml. A solution of 8AG of 100 times strength was made up in distilled water with the addition of a 1 M solution of NaOH drop by drop until it had all dissolved. The 100 times strength solution of 8AG was added to the culture medium after preliminary dilution 5 times with medium RPMI-1640. Mutants were selected in three stages: The cells were removed 3 times with selective medium and allowed to multiply 3 times on culture medium, after which they were transferred back again to the selective medium. The efficiency of selection was verified by transferring the mutant cells to culture medium containing hypoxanthine, aminopterine, and thymidine (HAT medium). This medium was prepared by adding a 100 times strength solution of thymidine ($1.6 \cdot 10^{-3}$ M) and hypoxanthine (10^{-2} M) and a 100 times strength solution of aminopterine ($4 \cdot 10^{-5}$ M) to the culture medium [4].

Viability was determined by counting the cells in a solution of trypan blue and eosin, made up in physiological saline buffered with phosphate buffer; the ratio of living (unstained) cells to the total number of cells, in percent, was estimated.

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EXPERIMENTAL RESULTS

During culture of the K-562 cells *in vitro* on medium containing 8AG a gradual decline in their viability was observed from 98 to 3-7%, with corresponding death of the cells during the first five passages (in the course of 17 days). The cells were then transferred to a culture medium in which they multiplied (viability rose to 95-99%). The selection procedure, as has been stated, was repeated 3 times. In the last stage the cells were maintained on medium with 20 μg/ml of 8AG for 14 passages in the course of 7 weeks; some of the cells were observed to die during the first six passages, and viability varied from 30 to 40% in different specimens. Starting with the 7th passage, viability of the clones rose sharply up to 91-98%. After the 14th passage the cells were washed and, after the last passage on culture medium, theywere transferred to HAT medium. Death of the cells on this medium was complete after three passages (in the course of 10 days). Original K-562 cells, very few of which died during this period in HAT medium (7-9% of cells died in different specimens), were used as the control. During selection and subsequent culture, mutant cells were successfully adapted to Soviet medium 199, thus greatly reducing the use of imported RPMI-1640 medium.

A new mutant cell line was thus obtained on the basis of human erythromyeloleukemia K-562.

This mutant cell line can be used for various purposes, such as to study the location of genes in human chromosomes. Prospects for the use of the new mutant line are linked with the position of K-562 cells in the erythromyelopoietic system. Line K-562 was obtained from the pleural fluid of a patient with a chronic form of myeloid leukemia [10]. Further information on the state and behavior of this cell line were given by the same authors 2 years later [11]. In particular, there are some interesting data on the morphological, cytogenetic, and immunologic characteristics of line K-562, as well as data confirming that this cell line is clearly differentiated. Possibly during adaptation and selection, the line was partially dedifferentiated. A number of experiments with the mutant cell line obtained by the present writer have shown that this line has lost the parental property of being a "unique target" for natural killers.

One of the prospects for the use of line K-562 and its mutant line now obtained is the breeding of a mutant cell clone on its basis that will be suitable for somatic cell hybridization.

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