Comparison of Cytostatic Sensitivities of L 1210 Cells and Human Stimulated Lymphocytes in Three Cell Proliferation Assays

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Summary. Three methods of measuring cell proliferation, viz., cellular ³H-thymidine uptake, counting of cells in suspension, and counting of colonies of cells grown in agar contained in glass capillaries, were compared by studying cell growth kinetics using the L 1210 cell line. We found the agar colony culture method to be most suitable and methodologically most advantageous. Using these cytokinetic models, we investigated the differential sensitivities of exponential and stationary phase L 1210 cells and normal, human, PHA-stimulated, peripheral T-lymphocytes to methotrexate, cytosine arabinoside, azathioprine, and a partially purified lymphocyte chalone preparation. L 1210 cells in exponential growth showed a higher drug sensitivity to all the agents tested than those in stationary growth. Normal, human T-lymphocytes exhibited less sensitivity to the tested agents. We found the agar culture to be more than twice as sensitive as the suspension culture and up to 8-fold more sensitive than the ³H-thymidine uptake method.

Key words: L 1210 – Cell growth kinetics – ³H-thymidine uptake – Suspension culture – Agar culture – Normal lymphocytes – Cytostatic agents.

Vergleich der Zytostatika-Empfindlichkeiten von L 1210-Zellen und menschlicher, stimulierter Lymphozyten in drei Zellproliferations-Assays

Lymphocyten waren im Vergleich dazu weniger empfindlich. Für die Bestimmung der unterschiedlichen Empfindlichkeiten der Zellen war die Agarkultur-Methode doppelt so empfindlich wie Zellzählung von Zellsuspensionen und bis zu achtfach empfindlicher als die Messung der $^3$H-Tdr-Aufnahme.


Principally, the various methods of measuring cell proliferation are direct modifications of three basic approaches: measurement of the incorporation of a radioactive precursor of DNA, RNA, or protein; measurement of the number of cells in suspension; and measurement of colony growth of cells seeded in a suitable matrix, such as agar or methylcellulose (Steel 1977). Each method has its restrictions and the choice of method must be done very critically with respect to the question being studied. The results obtained by testing a cell growth stimulator or inhibitor thus often depend on the method used (Meriwether and Bachur 1972). Unfortunately, few studies are known which directly compare these methods (Lord 1976). We, therefore, first studied the growth kinetics of a widely used, continuous cell line (L 1210) applying the three methods and established optimal test conditions for each method. We then followed the effects of three known inhibitors of cell proliferation and a natural inhibitor of lymphocyte proliferation on this cell line and on human, PHA-stimulated lymphocytes. Each method gave a different LD$_{50}$ value for each cytostatic agent, thus providing a means of evaluating the sensitivities of the methods used.

Materials and Methods

Cells and Culture Materials

The L 1210 cells (from Associated Biomedic Systems Inc., Buffalo, NY, USA) were cryopreserved in 10% dimethyl sulphoxide in liquid nitrogen. After thawing at 37 °C, stock suspension cultures were maintained in RPMI 1640 (Seromed GmbH, München, FRG) supplemented with 10% foetal calf serum (Seromed, GmbH München, FRG) and penicillin (100 units/ml), streptomycin (100 μg/ml) at 37 °C and 5% CO$_2$.

Human peripheral T-lymphocytes were isolated from the peripheral blood of healthy donors using Ficol Paque (Pharmacia Fine Chemical, Uppsala, Sweden) according to the method of Boyum (1968). The cells were pre-stimulated with PHA-M (Difeo Laboratories, Detroit, MI, USA) and assayed as described by Maurer et al. (1977).

Cell Growth Optimization

To determine the optimal inoculum for the suspension culture, L 1210 cells were seeded at densities ranging from $1 \times 10^3$ to $5 \times 10^3$ cells/ml in duplicate and counted after 5 days of incubation.

Population Kinetics

Over a period of 8 days total cell densities of cell suspensions (in duplicate; initial inoculum $5 \times 10^3$ cells/ml) were determined every 24 h. Simultaneously, 75 μl (in triplicate) of each cell suspension (adjusted to $1 \times 10^3$ cells/ml) were incubated in a microtitre plate with 20 μl (0.28 μCi) of a $^3$H-thymidine (Radiochemical Centre, Amersham, England) solution at 37 °C and 5% CO$_2$. After 90 min, the contents of each well were collected on glass fibre filters (Whatmann Grade GFA, W.&R. Balston, England) using a cell harvester (Otto Hiller Co., Madicon, WI, USA), washed twice with bidistilled water, dried at 50 °C.