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Protein degradation in human T-lymphocytes

E. Knecht, J. Renau-Piqueras, J. Hernández-Yago and S. Grisolía

Instituto de Investigaciones Citológicas de la Caja de Ahorros, Amadeo de Saboya 4, Valencia-10 (Spain), 28 July 1980

Summary. Protein from resting or phytohemagglutinin-stimulated human peripheral blood T-lymphocytes, pulse-labeled 'in vitro' for 1 h with ^{3}H-leucine, had a half-life of 30 h.

Small, nondividing lymphocytes have been used to study the activation process induced by mitogens because metabolic, functional and morphological parameters undergo large-scale changes[1]. Therefore, these cells also provide a convenient model to clarify the regulation of protein metabolism, because protein synthesis can be regulated in a precise way during activation. Although there has been considerable work on protein synthesis in lymphocytes[1], little has been done in the area of protein degradation, a process which is of equal importance with synthesis in maintaining and regulating the protein content of the cells[2]. The present paper describes the general characteristics of protein degradation in human T-lymphocytes.

Material and methods. Human peripheral blood T-lymphocytes were obtained as previously described[3]. Cell viability, checked by trypan blue exclusion, was over 95% and differential counts showed 96.0% T-cells. Cells were incubated at 37 °C in an atmosphere of 5% CO_2 in RPMI 1640 medium (Flow Lag, Irvine, Scotland) (10^6 viable cells/ml) with 10% foetal bovine serum 100 U/ml penicillin and 100 µg/ml streptomycin. For phytohemagglutinin (PHA) stimulation, T-cells were incubated at 37 °C for 30 h in the same medium containing 25 µg/ml PHA-P (Difco Lag, Detroit, Michigan, USA). To study the time course of the stimulation, T-lymphocytes cultures were incubated as described above for 6, 24, 48, 72 and 96 h. 1 h before ending the incubation period, cultures were pulse-labeled with [methyl-^{3}H]-thymidine (2 µCi/ml, 2 Ci/mmole). Autoradiographs were made, as previously described[4], by the 'stripping film' technique. Labeled and unlabeled lymphocytes were evaluated in 3 smears per case (300 cells/case). Lymphocytes from untreated chronic lymphocytic leukaemia (CLL) diagnosed as T-cell leukaemia were isolated and incubated under the same conditions as the normal lymphocytes.

For radioactive labeling, cells were incubated in medium containing L- [4, 5-^{3}H] leucine (0.05 mM, 52 Ci/mmole) for 1 h in siliconized Erlenmeyer flasks at 37 °C with shaking. Then, the cells were washed 4 times with fresh medium containing 2 mM unlabeled leucine with 5 min incubations at 37 °C between resuspension of the cells in medium and centrifugations at 300 × g. After washing, the cells were chased at 37 °C in fresh medium containing 2 mM L-leucine for 24 h. Aliquots of medium were removed at different intervals, cooled in ice and centrifuged (300 × g, 5 min) at 4 °C. The cell pellet was resuspended in phosphate buffered saline. Supernatants and cells were precipitated with 5% trichloroacetic acid (TCA). Precipitates were dissolved in 0.2 N NaOH. Radioactivity was determined by liquid scintillation counting. All counts were corrected for quenching using an internal standard. The TCA-soluble radioactivity of supernatant and cells at each time-point was calculated as the difference between the value at each chase time and zero time and expressed as percent of total radioactivity (TCA-soluble plus TCA-insoluble radioactivity of both cells and supernatant). The half-lives were estimated from the slopes of the best fitted regression lines when the degradation curves were plotted semilogarithmically[5].

Results and discussion. Protein synthesis in resting T-lymphocytes is minimal, perhaps reflecting only the turnover of cell components. To study the mechanism/s of protein degradation in these cells, we have determined first their rate of protein degradation and then compared it with that found in CL (resting) and in PHA-transformed T-lymphocytes. A 30 h PHA incubation period was chosen so that most cells were in the G_1 phase (figure 1).

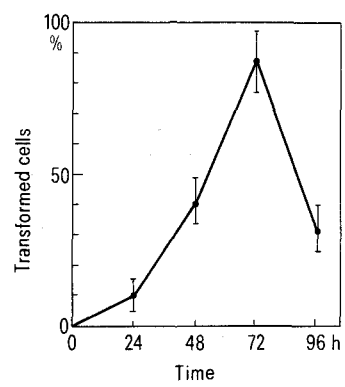

Fig. 1. Time course of T-lymphocyte stimulation by PHA.

To ascertain rates of protein degradation it is necessary to minimize reutilization of the isotope[6]. The use of serial extraction eliminates radioactive precursor, especially that appearing in the medium early in the chase period. Moreover, since all experiments were conducted using a large volume of fresh medium relative to cell volume and containing 2 mM L-leucine, reutilization should have been minimal.

Figure 2 shows the kinetics of protein degradation for a representative experiment. At zero time TCA-soluble radioactivity was only 9.6% of total radioactivity. TCA-soluble radioactivity in the cells remained at a fairly constant low level (about 5% of total radioactivity) throughout the experiments, while TCA-soluble radioactivity in the medium accumulated with the chase time. Degradation was approximately exponential with indication of an early phase of fast degradation. The half-lives of the labeled proteins were comparable in resting, PHA-transformed and CL T-lymphocytes: 31.1±6.7, 39.4±7.3 and 27.8±5.8 h respectively. These values are approximately twice the half-life calculated by others for rat lymphocytes using radioautography and grain counting[6]. This discrepancy could possibly be due to species differences in turnover rate, but is more likely to be due to the fact that labeling of rat lymphocytes was carried out 'in vivo', whereas in our experiments labeling was carried out 'in vitro', which, surprisingly, may be more accurate. This is because after i.v. injection of an isotope, there is a preferential labeling of the lymphocytes from peripheral blood, a relatively small pool which is subsequently diluted by the less labeled lymphocytes from spleen, thymus, thoracic duct and other lymphoid systems, thus causing an erroneaous overeste of the degradation rate. Since the life-span of lymphocytes is very long[7], it can be concluded that virtually all protein turnover in human T-lymphocytes is intracellular rather than involving death and renewal of entire cells.

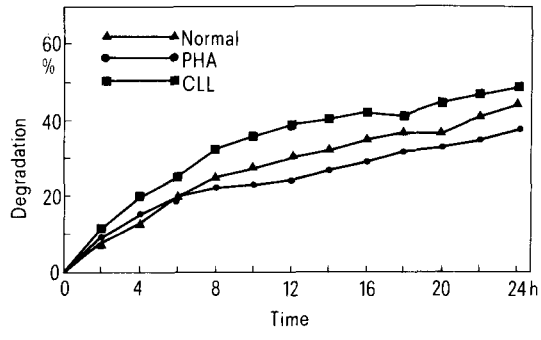

Fig. 2. Kinetics of protein degradation in normal, chronic leukaemic and PHA-transformed T-lymphocytes. Viability of the cells at the end of the experiment was 84.0±9.1, 89.3±7.5 and 86.4±9.1 respectively.

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Correction of the anomalous electrophoretic behavior of ribonuclease A[1]

J.M. Thomas and M.E. Hodes

Department of Medical Genetics, Indiana University School of Medicine, Indianapolis (Indiana 46223, USA), 13 October 1980

Summary. Ribonuclease A behaves anomalously on polyacrylamide gel electrophoresis at acid pH. The distance traveled by the protein is a function of the amount of enzyme added at the lower range of detectable activity (10 pg to 100 ng). Addition of myoglobin (1 mg/ml) abolishes the anomaly. The observations are consistent with the known affinity of RNase for anions. Caution is warranted in the interpretation of apparent electrophoretic variants of RNase observed at low concentrations of enzyme.

Implicit in the use of electrophoretic techniques for comparing different proteins is the assumption that for a given set of experimental conditions (e.g., supporting medium, buffer, ionic strength, pH, applied voltage and time), a given protein will always migrate the same distance relative to a reference. Relative migration distance (and mobility) is also assumed to be independent of the amount of a given protein subjected to electrophoresis. Deviations from the latter assumption appear to have been rarely observed for any protein and never reported for ribonuclease A (RNase A) from bovine pancreas. We report here that the mobility of RNase A in a common acidic buffer system during polyacrylamide gel electrophoresis (PAGE) is a function of the amount of protein analyzed and that the anomalous electrophoretic behavior at low levels of protein can be rectified by the addition of another polycationic protein, myoglobin. These observations are consistent with the known affinity of RNase A for anions.

Materials and methods. RNase A was purchased as ribonuclease I 'A' from Miles Laboratories, Inc., Elkhart, IN. Myoglobin and bovine serum albumin from sperm whale skeletal muscle were from Sigma Chemical Company, St. Louis, MO.

RNase zymogram. After electrophoresis, the polyacrylamide gel (see legend for fig.) was equilibrated in 0.05 M sodium phosphate buffer, pH 8.0. A 1% agarose gel 0.79 mm thick containing 2 mg/ml RNA in the phosphate buffer was placed over the polyacrylamide gel. After 20 h at