Degradation of Rapidly Labelled RNA in Proliferating and Non-Proliferating Human Acute Leukemia Cells

In proliferating cells, rapidly labelled RNA is generally made up of ribosomal, and, to a lesser degree, messenger fractions whose stability can be evaluated by the inhibition of further synthesis with actinomycin D. With this method numerous workers[1-7] have found 2 metabolically different RNAs, the former highly unstable, the latter relatively more stable. A wide range of differences in cell unstable RNA content has been observed, coupled with a significant dependence on the degree of cell maturity or of proliferative activity[4,6].

Previous investigations have shown similar breakdown patterns in acute leukemia blasts and normal hemopoietic cells[8]. Since the acute leukemia cell population has 2 compartments, i.e. proliferating and non-proliferating, these being 2 evolutional aspects of the same cell line[9-14], it was considered that RNA breakdown behaviour in these compartments might point to a relationship between proliferative activity and the metabolic behaviour of RNA.

Materials and methods. Our series consisted of 4 cases of acute myeloblastic leukemia (cases 1, 2, 3 and 4), 1 case of acute lymphoblastic leukemia (case 5) and 1 case of acute monoblastic leukemia (case 6). In all cases bone marrow blood was withdrawn in a heparinized syringe and diluted in Hanks' liquid (1:1 or 1:4) and horse serum was added to make a final concentration of 20%. Incubation was carried out in rotating system at 37 °C.

RNA turnover was studied in the following manner: the cells were exposed to uridine-5-H_3 (sp. act. 24.4 c/m*M*) at a concentration of 10 μc/ml of culture for 1 h. They were then transferred to a medium containing actinomycin D (supplied by Merck, Sharp and Dohme) at a final concentration of 10 μg/ml. Smears were made at various intervals for 5 h. The degree of inhibition of the synthesis of RNA induced by actinomycin D was evaluated by incubation for 10 min with this compound (10 μg/ml) followed by the addition of uridine-5-H_3 (10 μc/ml) for 1 h.

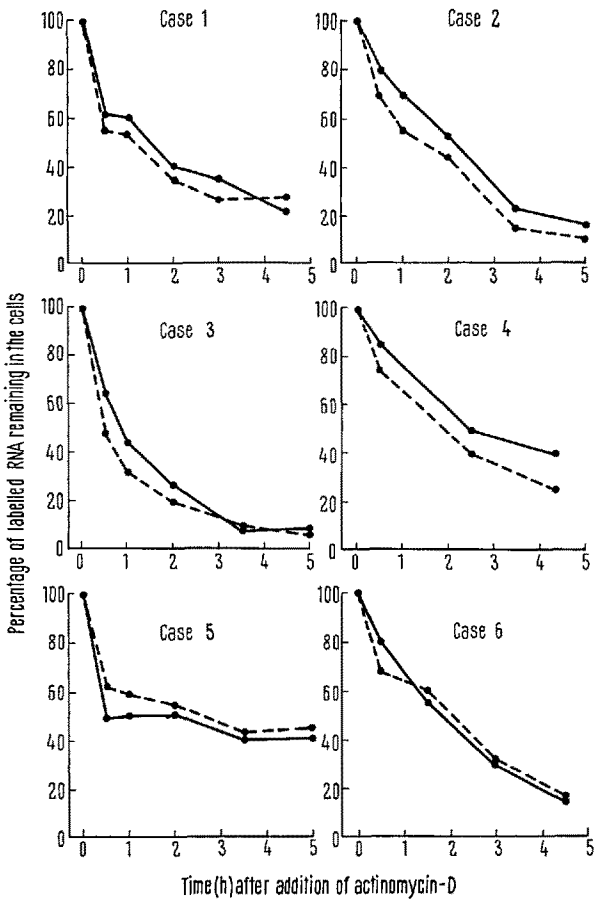
Cell proliferative activity was studied in bone marrow and peripheral blood. After dilution in Hanks' liquid, this was exposed to thymidine-H_3 (sp. act. 17 c/m*M*) at a concentration of 2 μc/ml for 1 h in a rotating system at 37 °C.

After fixing in methyl Carnoy, the smears were immersed in Ilford-K_2 emulsion previously diluted 1:1 with distilled water and left exposed for 5–15 days at 4 °C. They were then developed and fixed, and counterstained with May-Grünwald-Giemsa stain buffered to pH 7.4. The allocation of blasts to the proliferating or non-proliferating compartment was based on their diameter and on a parallel study (using thymidine-H_3) of their proliferative activity.

The percentage of cells labelled was regularly ascertained for at least 500 cells in each slide.

Results. In all 6 cases, rapidly labelled RNA degradation revealed 2 metabolically different RNAs, one very unstable (breakdown within 2–3 h), the other relatively more stable. The % fall of grains in function of time was not significantly different in the proliferating and non-proliferating compartments, showing that identical metabolic behaviour was present in both compartments in all our acute leukemia cases (Figure).

Discussion. Since rapidly labelled RNA consists of both ribosomal and messenger fractions, the half-life value



Breakdown of labelled RNA in proliferating (——) and non-proliferating (– – – –) acute myeloblastic leukemia cells (cases 1–4), acute lymphoblastic leukemia cells (case 5) and acute monoblastic leukemia cells (case 6).

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estimated from RNA turnover values is naturally only the mean between the 2 fractions. SINKS and HAYHOE observed greater RNA stability in actively proliferating phytohemogglutinin-stimulated lymphocytes by comparison with non-proliferating chronic lymphocytic leukemia cells[3]. They consider that these differences simply reflect basal differences in RNA patterns during the various stages of the cell cycle. Our present finding of similar RNA breakdown rates in proliferating and non-proliferating cells in acute leukemia, however, indicates that loss of proliferative activity does not necessarily involve significant changes in RNA turnover.

STORTI and TORELLI, working with normal bone marrow erythroid and myeloid cells, found clear RNA instability in immature cells and greater stability in the advanced stages of both cell lines[4-6]. Similar turnover rates in both compartments in acute leukemia show that the transformation from large to small blast, with loss of proliferative activity and without differentiative evolution[9-11,13], is not accompanied by the formation of the 'metabolically stable' RNAs typical of the more advanced stages of erythroid and myeloid maturation.

The following conclusions may be drawn: (1) there are no significant differences in RNA breakdown in proliferating and non-proliferating blasts in acute leukemia; in each case, the half-life range is 1.5–3 h; (2) acute leukemia blast cells, even if in an advanced stage of evolution, are not capable of synthesizing the so-called 'stable' RNAs found in the more advanced stages of erythroid and myeloid maturation[15].

Riassunto. In cellule di leucemia acuta umana incubate in vitro viene osservata una rapida degradazione dell'ARN sintetizzato prima del contatto actinomicinico e vengono distinte 2 varietà di RNA a differente labilità metabolica. Uno stesso comportamento metabolico è stato osservato negli elementi blastici proliferanti e nonproliferanti.

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Mécanisme d'action du chloramphénicol dans l'inhibition de la production d'anticorps induite par de différents types d'antigènes

Le rôle fondamental des acides nucléiques dans la synthèse des anticorps est presque universellement admis[1-6] et cela est encore démontré par l'action de certaines substances (qui agissent sur le métabolisme des acides nucléiques) sur la synthèse des anticorps. Parmi les substances les plus connues à ce titre il y a quelques antibiotiques tels la puromycine, l'actinomycine D et le chloramphénicol: le mécanisme d'action des 2 premières substances est suffisamment connu[7-9]; il y a, en revanche, de nombreux points d'incertitude pour ce qui concerne l'action du chloramphénicol: en effet, AMBROSE et COONS pensent que le chloramphénicol inhibe la synthèse de l'ARN messager[10,11], WEISBERGER et coll., en revanche, pensent que l'ARN responsable de l'élaboration du modèle des anticorps se forme malgré la présence du chloramphénicol, mais qu'il est inhibé dans sa fonction spécifique parce qu'il est empêché d'entrer en contact avec les nucléoprotéines des ribosomes qui contrôlent la synthèse protéique[12-14]. Dans le présent travail nous avons essayé de définir le mécanisme d'action du chloramphénicol et surtout de définir à quel stade de la synthèse des anticorps intervient l'inhibition.

On a employé des lapins sélectionnés du poids moyen de 2 kg maintenus à un régime normalisé. Les antigènes étaient: albumine et globulines bovines (SAB, SGB), globules rouges (GR) de rat et de cobaye (en suspension à 25%) et 2 souches de *Escherichia coli*, l'une sensible au chloramphénicol, l'autre résistante (détermination selon la méthode des disques) employées en suspension à 2%. Pour immuniser les lapins on faisait 4 injections d'antigène par voie i.v. ou s.c. à 7 jours l'une de l'autre. Les quantités injectées étaient, respectivement, de 10 mg pour SAB et SGB, de 2 ml pour GR et pour chacune des suspensions bactériennes. Dans chaque expérience, un lot d'animaux non traités par le chloramphénicol servait de témoin. L'administration de l'antibiotique commençait 24 h avant le début de l'immunisation et se poursuivait avec 2 injections journalières de 0,5 g, le produit employé était du chloramphénicol succiné (CAF) fourni par l'Istituto Sieroterapico Milanese.

Les titres des anticorps étaient déterminés dans le sérum avant chaque injection et, à la fin du traitement, 4 jours après la dernière injection: ces déterminations étaient effectuées selon les techniques habituelles de l'agglutination pour les antigènes corpusculaires et selon celles de la précipitation zonale pour les antigènes solubles.

De chaque groupe d'animaux, à la fin de l'immunisation, on extrayait l'ARN du sérum selon une technique décrite dans un travail précédent[6]. Pour induire l'apparition d'anticorps actifs sur les mêmes antigènes avec lesquels avaient été immunisés les animaux des quels on avait extrait l'ARN, ce dernier était injecté soit à des

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