surface (data not shown); however, unlike binding of rabbit IgG to proximal enterocytes, this occurred as readily at pH 7.2 as at pH 6.0 and could not therefore be taken as indicative of binding to comparable Fc γ receptors, although as with binding to proximal small intestine it was Fc γ dependent. Actin, present in enterocyte terminal web regions, has also been suggested to be capable of binding IgG in an Fc-dependent manner[21] and could have been exposed during the culture period.

The effect of dexamethasone in causing a precocious loss of Fc γ receptor when administered to suckling rats and an apparent increase or at least maintenance in expression when added to cultured foetal gut, is comparable to the effect of glucocorticoids on lactase activity. Thus administration of corticosteroid to suckling rats causes a precocious decrease in lactase in enterocytes (and a concomitant rise in sucrase and maltase)[22], whilst addition of dexamethasone to cultured foetal rat intestine causes an increase in lactase activity compared to controls[16]. Assessment of Fc γ expression in the method we have used cannot be quantified and we do not know whether Fc γ receptors were present on enterocytes at the start of culture of the tissue and were then maintained in expression by dexamethasone, or whether dexamethasone induced them to appear. Dexamethasone has been suggested to have a protective effect in maintaining enzyme activity in cultured 4-day-old post-natal rat gut with respect to lactase, and a promoting effect with respect to maltase and sucrase[23]. Now that the Fc γ receptor has been isolated from suckling rat jejunal enterocytes, and monoclonal antibodies prepared against it[24], more accurate measurement of hormone-induced expression of Fc γ receptors could be made. Our preliminary findings do however point to their expression being controlled by glucocorticoids in a manner similar to that of certain brush border enzymes, and as part of an overall pattern of development of the gut that adapts to a post-weaning diet[25].

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Proliferation and cell loss of human leukemic cell subpopulations in liquid culture

P. A. Bernabei, F. C. Agostino, R. Bezzini, R. Saccardi, V. Gattei, V. Santini, M. Casini and P. Rossi Ferrini

Div. Ematologia, USL 10/D-Universita' degli Studi, Policlinico di Careggi, I-50134 Firenze (Italy)
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Summary. A kinetic study was performed on leukemic blasts from patients with acute myeloid leukemia, separated into 2 subpopulations by a specific density gradient. The growth curve and the [^{3}H]-thymidine uptake were simultaneously analyzed. While cumulative nucleotide uptake fitted with the growth kinetics in the low-density fraction, such a concordance was not found in the high-density subpopulation. That indicated the occurrence of simultaneous growth and loss in the high density fraction, which could not be evaluated by a simple numerical determination.
Key words. Acute myelogenous leukemia; cell subpopulations; cell loss; kinetics in culture.

Leukemic cells harvested from patients with acute myeloid leukemia (AML) may display heterogeneous features as to karyotype[1], phenotype[2] and cytokinetics[3]. According to the difference in size, leukemic cells can be subdivided into two subpopulations which show different cytokinetic patterns: the large size subpopulation, which apparently includes the proliferative component, and the small cell subpopulation, which is quiescent[4] and possibly responsible for the kinetic resistance to chemotherapy[5]. This subdivision has also been supported by sedimentation and elutriation studies, showing that the cells characterized by high or low density display a high or a low labeling index, respectively[6, 7]. This has resulted in most kinetic studies being focused on low-density cells, disregarding the high-density subpopulation.

We considered further study of this topic worthwhile, trying to resolve the in vitro cytokinetics of low- and high-density leukemic cell subpopulations by measuring both cell proliferation and loss. For this purpose we used the method proposed by Olivotto et al.[8], based on the simultaneous determination of the cell number kinetics and of the time-course of the rate of [^{3}H]-thymidine ([^{3}H]-Tdr) incorporation into DNA (R(t)) of the cell population under study. These authors showed that within any cell population, when the mean time spent in S by the cells remains constant, the integral of R(t) as a function of time reflects the total cell number increase if, and only if, the cell loss is negligible, according to the following equation:

$$\int_0^t R(t)\,dt = k \times (N_t - N_0) \qquad [1]$$

where $N_t - N_0$ is the cell number variation in the interval 0/t and k corresponds to the amount of [^{3}H]-Tdr expressed in DPM, incorporated by each cell which has passed through S. On the other hand, when the cell loss is substantial, it can be estimated by equating $\int_0^t R(t)\,dt$ with the kinetics of the cell number increase, according to the equation:

$$\int_0^t R(t)\,dt = k \times [(N_t - N_0) + CL] \qquad [2]$$

where CL = number of cells lost up to time t.

Materials and methods. Mononucleated cells from peripheral blood of 6 untreated patients affected by AML, selected for the high percentage of circulating blasts (at least 90%), where applied to a BSA density gradient at a specific density of 1.062 g/ml, and centrifuged for 30 min at 2000 g. Two cellular subpopulations were collected, one characterized by a specific density $\leq$ 1.062 g/ml (Sp 1), the other $>$ 1.062 g/ml (Sp 2).

Cells from both fractions were washed twice, counted and seeded at a concentration of 1.10^5/ml in multiwell plates (1 ml of cell suspension per plate) and in microwells (0.2 ml of cell suspension per well) in culture medium containing RPMI 1640 with 15% fetal calf serum and 10% GCT conditioned medium[9]. Cultures were then incubated at 37 °C in a fully-humified atmosphere with 5% CO_2 for 7 days. Over this period no refeeding of the cultures was performed. The number of viable cells in culture was determined daily by counting, in quadruplicate, trypan blue negative cells collected from multiwell plates. From the same samples slides stained with Giemsa and Sudan black and esterases were prepared by a cytocentrifuge. Each well was used only for one determination.

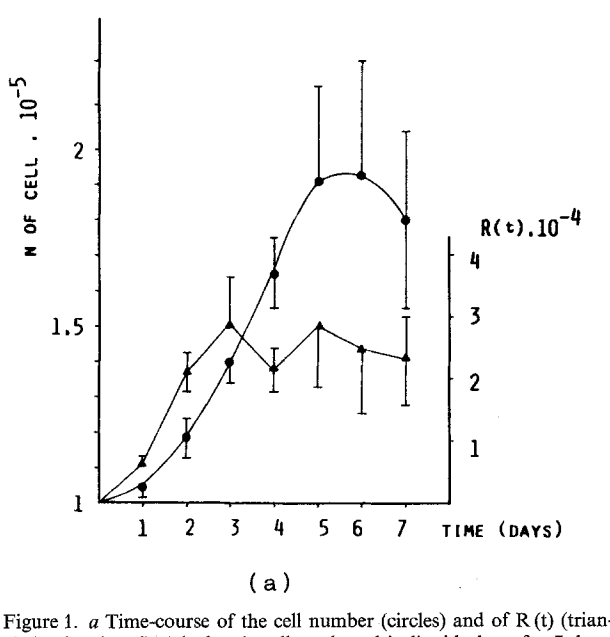
R (t) was evaluated daily by 1-h pulse-labeling of 4 microwells with [^{3}H]-Tdr (sp. act. 5 Ci/mmol; 2.5 μCi/ml of cell suspension). The cellular contents of the pulsed wells were collected by a multiple cell harvester on glass-fiber filters and the radioactivity incorporated by the cells was measured by scintillation counting (DPM), with 45% efficiency.

$\int_0^t R(t)\,dt$, was calculated by integrating the area underneath the time course of R (t) according to the method proposed by Olivotto et al.[8]. The observation of cultured cells stained with Giemsa and Sudan Black, as well as the esterases reaction confirmed the leukemic morphology of the cells of both fractions throughout the incubation, virtually excluding contamination with normal nucleated cells.

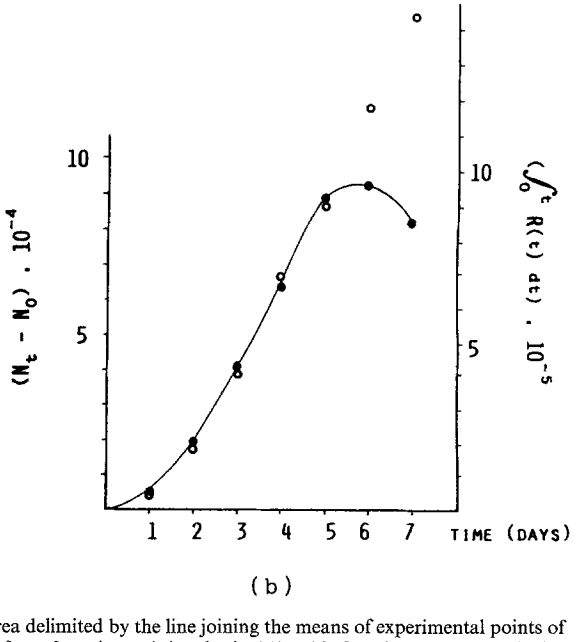
Results and discussion. In figure 1a the time-course of the total cell number in Sp 1 is represented by a sigmoid curve which reaches a plateau at day 5. Figure 1a also shows that the time-course of R (t) of Sp 1 displays an increase up to day 3 and a subsequent slow decrease. The cell number increases, $N_t - N_0$ and $\int_0^t R(t)\,dt$, computed from data in figure 1a, are compared in figure 1b. It is evident that the kinetics of these two parameters practically coincide up to day 5, so that, up to this time, the equation [1] is verified, with k = 11.04.

After day 5 the $\int_0^t R(t)\,dt$ keeps increasing while the $N_t - N_0$ reaches a peak and then starts declining. According to the analytical method adopted, this means that up to day 5 cell loss is negligible in Sp 1, becoming substantial after this period. Assuming that throughout incubation $\int_0^t R(t)$ is directly proportional to the total number of cells generated in vitro, $[(N_t - N_0) + CL]$, the real increase can be estimated at any time by: $\int_0^t R(t)$, according to the eqn [2]. On this basis it is possible to calculate, from figure 1b, that the actual number of cells generated in Sp 1 over the total incubation time is about 140,000/ml. Recalling that the inoculum in this experiment was 100,000 cells/ml (fig. 1a), it is possible to infer that the initial population underwent no more than a 1.4-fold increase in 7 days. Since the longest mean generation time so far reported for leukemic cells is 47 h[10] only a minority of the cells belonging to the Sp 1 apparently underwent replication in vitro.

Figure 2a shows the time-course of total cell number and R (t) relative to Sp 2. This subpopulation, although charac-



(a)



(b)

Figure 1. *a* Time-course of the cell number (circles) and of R (t) (triangles) related to Sp 1 leukemic cells, cultured in liquid phase for 7 days. *b* Time-course of $\int_0^t R(t)\,dt$ in Sp 1 (open circles), obtained by integrating the area delimited by the line joining the means of experimental points of R (t), from 0 to time t (triangles in (*a*)). This function is compared with $N_t - N_0$ (closed circles) computed from (*a*). Each point represents the mean of six experiments. Vertical bars represent SEM.

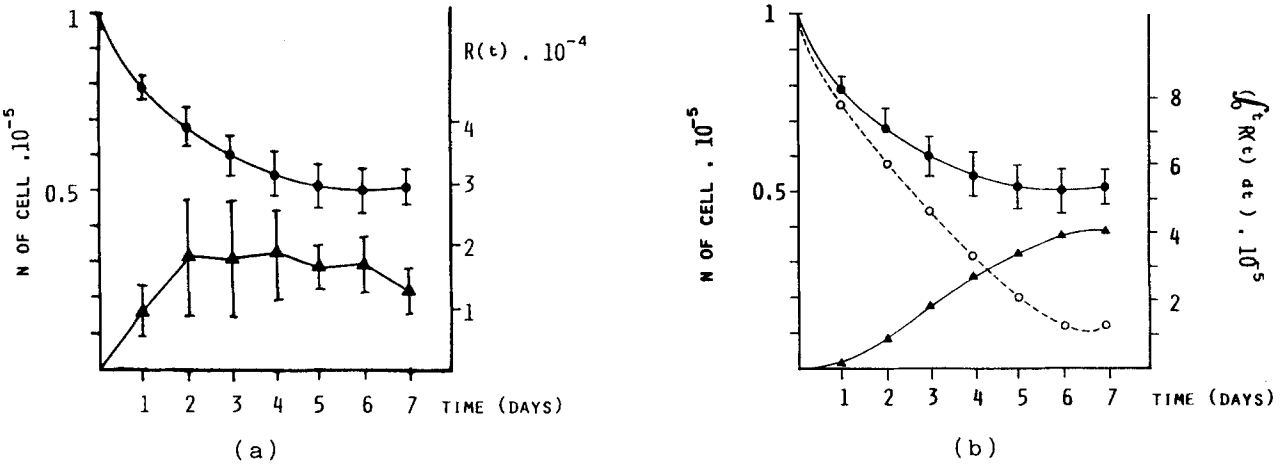


Figure 2. *a* Time-course of the cell number (circles) and of R (t) (triangles) related to Sp 2 leukemic cells, cultured in liquid phase for 7 days. *b* Time-course of the cell number decrease (closed circles) and of $\int_0^t R(t)\,dt$ (triangles), related to leukemic cells from Sp 2, cultured in liquid phase for 7 days. Each point represents the mean of six experiments; the vertical bars represent SEM. The graphic representation of $\int_0^t R(t)\,dt$ could represent also the numerical increase in Sp 2 disguised by a loss greater than that which was experimentally determined. The open circles represent the evaluation of the real loss, performed by subtracting, point by point, the actual increase from the experimental loss.

terized by a cell loss during incubation, shows a substantial [3H]-Tdr uptake up to day 7, indicating the presence of a not negligible number of cycling cells in this fraction. Applying to the Sp 2 the same mathematical procedure used for the data in figure 1, it is possible to calculate the actual growth kinetics of this fraction and, using the same k obtained for Sp 1, it is now possible to resolve the kinetics of the total cell number in Sp 2 into the kinetics of the real cell increment and cell loss as shown in figure 2b.

Based on this data we conclude that Sp 2, far from being devoid of cells endowed of proliferative capacity, includes a cohort of cells which re-enter the cycle and undergo an expansion not dissimilar to that of the Sp 1. Thus the main difference between Sp 1 and Sp 2 is not the presence of proliferating cells, but the amount of cell loss, which is much larger in Sp 2. This conclusion, which stresses the importance of evaluating leukemic subpopulation kinetics by means of a method which takes into account both cell proliferation and cell loss, should be borne in mind in attempts to set up cytokinetic systems capable of analyzing the effects of drugs or biological factors involved in the control of human leukemic growth.

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Aneuploidy of glandular epithelial cells in histologically normal prostate glands

T. I. Malinin[a], F. J. Hornicek[a], N. L. Block[b] and G. I. Malinin[c]

[a] *Surgical Research Laboratories, Department of Surgery, and* [b] *Department of Urology, University of Miami School of Medicine, Miami (Florida 33101, USA), and* [c] *Department of Physics, Georgetown University, Washington (D.C. 20057, USA)*
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Summary. The percentage of aneuploidy in normal prostate glands from subjets 13–38 years old and 45–66 years old ranged from 0–78% and 0–63%, respectively. In contrast to adults, aneuploidy was absent in fetal and postnatal prostates. It is concluded that aneuploidy is a fundamental attribute of histologically normal adult prostate glands.
Key words. Prostate; aneuploidy; flow cytometry.