

CELLULAR KINETICS IN ACUTE LEUKEMIA¹

ALVIN M. MAUER, M.D. AND BEATRICE C. LAMPKIN, M.D.

Department of Pediatrics, University of Cincinnati, Cincinnati, Ohio 45221, and The Children's Hospital, and The Children's Hospital Research Foundation, Cincinnati, Ohio²

ABSTRACT

In most patients with acute leukemia, the generation time of leukemic cells is about 60 hours, longer than for normal cells. Approximate times for the phases of the cell cycle are: DNA synthesis (S), 20 hours; mitosis (M), 2 hours; and the post-synthesis and post-mitosis rest phases (G₂ and G₁), 2 and 36 hours, respectively. A most important finding has been that a variable proportion of leukemic cells are out of cycle, that is, are in a resting or G₀ state. These resting cells are in equilibrium with the dividing cells, and some, as yet unknown, control mechanism for leukemic-cell growth controls the flow of cells from one compartment to the other. A critical feature of the resting cells is that they are relatively resistant to cycle-dependent chemotherapeutic agents.

Much information has been obtained concerning the effects of drugs on the proliferative characteristics of leukemic cells. This information provides the basis for designing regimens with better timing of drug administration and advantageous use of combined chemotherapy.

INTRODUCTION

A favorite ploy of history teachers is to reveal to their students that the Holy Roman Empire was badly misnamed because it was not holy, Roman, nor an empire. In a similar vein, for generations, physicians have conceived of the leukemic process as one of rapid, uncontrolled proliferation of abnormal cells. During the past few years, however, it has become apparent that this concept of malignant cell growth was likewise erroneous. From the kinetic studies of leukemic cells to be discussed in this paper, the proliferative rate is slower than that of normal cells, and there is evidence for a rather sophisticated system of growth-regulatory mechanisms.

Early indication that leukemic-cell growth rates are less than for normal blood-cell precursors was presented by Astaldi and Mauri (1953). With the advent of radioisotopically labeled DNA and RNA precursors shortly thereafter, methods became available for specific studies of leukemic-cell proliferative characteristics. As knowledge of these characteristics has accumulated, attempts to correlate chemotherapy and cell kinetics have been possible. Information concerning the pathophysiology of the leukemic process has become available. Of particular value have been the concepts derived from kinetic studies which have application for design of chemotherapeutic regimens. Therefore kinetic studies of acute leukemia have been of value as one of the several possible approaches to an understanding of the disease process.

METHODS OF STUDY

The earliest possible method of study was the measure of the number of mitotic figures present in the leukemic-cell population in comparison to that of normal dividing cells (Astaldi and Mauri, 1953). The measure of mitotic indices (Japa, 1942) continues to be of value in judging overall proliferative activity and response of the leukemic cells to chemotherapy.

The advent of labeled DNA and RNA precursors added a new dimension to the study of cell proliferation. Most commonly used is ³H-thymidine (³HT)

¹This study was supported by Research Grant CA 04826 and Training Grant CA 05196 from the National Cancer Institute and a grant from the American Cancer Society. Dr. Lampkin is a Leukemia Scholar of the Leukemia Society of America.

²Requests for reprints should be addressed to: Alvin M. Mauer, M.D., The Children's Hospital, Cincinnati, Ohio 45229.

which specifically is incorporated into DNA only during the DNA synthetic phase of the cell cycle and remains with the cell in the nuclear DNA (Hughes, 1970). A labeling index with ^3HT obtained by autoradiography therefore gives an indication of the percent of cells in DNA synthesis within the cell population, another measure of overall proliferative activity. Another assessment of DNA synthetic activity within a cell population can be obtained by measuring the incorporation of ^3H - or ^{14}C -labeled thymidine into the DNA by means of liquid scintillation counting techniques (Lampkin, *et al.*, 1971).

An important consideration is the time required for the various phases of the cell cycle: mitosis (M); the interphase between mitosis and DNA synthesis (G_1); DNA synthesis (S); and the period between S and M (G_2). The total time required from one mitosis to a subsequent mitosis is the generation time. Another measure which is sometimes used is the time required for a dividing population to double in number. If all cells divide in a similar fashion within the population and no loss of cells occurs, the doubling time and generation time are the same. In most cell populations, however, not all cells are engaged in proliferative activity and some loss of cells does occur. Therefore in most situations the population-doubling time is slower than the cell-generation time.

Recently it has been possible to determine the content of cellular DNA by microspectrophotometry, a technique that has been used in some studies of leukemic-cell kinetics (Ernst and Killman, 1970). Coupled with ^3HT labeling and autoradiography, the technique can give information about the distribution of cells within the DNA synthesis phase for cells just beginning the phase, with nearly normal DNA content, to cells almost finished the phase, with twice normal DNA content. Cells in G_2 are found to have twice normal DNA content, but are unlabeled because DNA synthesis has been completed. This time-consuming type of study is useful for some special situations.

Several methods have been used to assess the timing of the generation cycle. The most difficult has been the observation of labeling in mitotic figures after a single *in vivo* injection of ^3HT . The label is incorporated rapidly into nuclei of cells in DNA synthesis and is available for only about 20 to 30 minutes (Rubini, *et al.*, 1960). Thus, "flash" labeling is obtained. The labeled cells, representing the cohort in DNA synthesis at the time of injection, subsequently complete DNA synthesis and proceed to the following phases of the generation cycle. This progress can be followed by serial samples of the cell population, by means of bone-marrow aspiration in the case of acute leukemia. A clear marker for observing the progress is the appearance and disappearance of the labeled cells in the mitotic phase, where the labeled cells can be distinguished by their altered appearance. The time for appearance of labeled mitotic figures represents the time needed to go through G_2 . The time from mid-point of appearance to mid-point of disappearance of the labeled mitotic figures represents the time required for DNA synthesis. When the next wave of labeled mitotic figures appears, the generation time for these cells is indicated.

Another method of measuring generation time that has been used has been the serial determination of grain counts in labeled cells. The label, once incorporated, becomes diluted in daughter cells by one-half during each mitotic division (Cronkite, *et al.*, 1961). Therefore, by determining the half-time for decreasing mean grain counts, the generation time should be possible to measure. Unfortunately, in practice, this method is not suitable. There is some label reutilization and furthermore some entry of cells into a prolonged rest phase (G_0), which affects the rate of label concentration decrease in the cell population.

A more rapid measure of DNA synthesis time can be obtained with a double-labeling technique. By this method, ^3HT is injected *in vivo* and at some time thereafter marrow is aspirated and incubated with ^{14}CT *in vitro*. Cells labeled with ^3HT and ^{14}CT can be distinguished by special autoradiographic techniques.

From the ratio of ^3HT -labeled cells to doubly labeled cells and the elapsed time between labeling procedures, the DNA synthesis time can be determined.

Effects of chemotherapeutic agents on proliferative activity can be measured by obtaining serial determinations of labeling indices and mitotic indices following administration of the agent. In some special situations, such as the study of methotrexate effect, two labels can be used. In this case, ^3HT will be incorporated by affected cells because this compound is beyond the metabolic block imposed by the drug. Thus cells capable of DNA synthesis but blocked by methotrexate will be demonstrated. On the other hand, ^3H -deoxyuridine (^3H -dU) will not be incorporated by affected cells, because the block prevents production of the thymidine needed for DNA synthesis from this precursor substance. Thus the degree and duration of the methotrexate effect will be determined with the label.

GENERAL FEATURES OF LEUKEMIC-CELL KINETICS

Several recent review articles giving details about cell kinetics in acute leukemia are available (Cronkite, 1967; Killman, 1968). From studies of the generation cycle with the labeled mitotic-index technique, the times for cell division are determined to be longer than for normal hematopoietic cells (Saunders, *et al.*, 1967; Wagner, *et al.*, 1972). The generation time is in the range of 50 to 70 hours, with S about 20 hours, G_2 about 2 hours, M about 2 hours, and G_1 about 30 to 35 hours. Some variation from patient to patient has been observed in these times.

From the earliest studies of the patterns of cell proliferation in acute leukemia, there has been indication that the cell population is not uniform with respect to proliferative activity. Labeling indices with ^3HT indicated that leukemic cells in blood had a lesser degree of proliferative activity than did the bone-marrow-cell population (Mauer and Fisher, 1962). Even in the bone marrow, there are two functionally different cell populations discernible (Gavosto, *et al.*, 1967; Mauer and Fisher, 1966; Killman, 1965). One population of larger cells with fine nuclear chromatin is the proliferative compartment. The other, comprised of smaller cells with dense nuclear chromatin, is a compartment of cells exhibiting no proliferative activity. The smaller cells arise from the larger cells, as some of these cells drop out of the proliferative cycle to become resting cells.

It was observed by Gavosto and co-workers (1967) that the larger cell compartment was non-self-maintaining, in that the loss of cells to the resting compartment was greater than the birth rate of larger cells. Therefore a source of cells coming into proliferative activity had to be found. Subsequently, it was demonstrated that small, resting leukemic cells were capable of reentering the proliferative phase and thus could serve as a stem-cell compartment for the larger cells (Saunders and Mauer, 1969). The cell population was in a dynamic equilibrium between resting and proliferative phases. The resting cells would then be only temporarily out of phase (in G_0). This population of resting cells, less sensitive to phase-dependent chemotherapeutic agents but capable of return to proliferative activity, has obvious importance for design of treatment regimens. Furthermore, in considering the pathogenesis of acute leukemia, these findings are compatible with a single cell mutation forming a self-maintaining clone of malignant cells.

EVIDENCES OF CONTROL MECHANISMS FOR LEUKEMIC-CELL GROWTH

One of the earliest indications that some control over leukemic-cell proliferation exists was the observation that proliferative activity was similar in various marrow sites sampled either simultaneously (Mauer and Fisher, 1966) or during different times of the day (Saunders, *et al.*, 1967). This finding would suggest that systemic humoral substances, not local environmental conditions, were probably responsible for proliferation control (Mauer, *et al.*, 1972).

It was also observed that considerable variation in proliferative activity occurs from patient to patient, and even in the same patient during different phases of his disease (Foadi, *et al.*, 1967; Pileri, *et al.*, 1967; Saunders, *et al.*, 1967). In general, the least degree of proliferative activity occurs at the time of diagnosis, when, from duration of symptoms, one would expect the longest growth period for the cell population to have been possible. On the other hand, when the population is studied in a subsequent relapse, proliferative activity of the cells is greater. At this time, the duration of symptoms, if any, is short, and it would be expected that the duration of population growth is also short. The proliferative activity varies inversely with the number of resting leukemic cells.

The most probable explanation for these findings is that, with progressive duration of growth, there is an increasing number of cells entering into the resting phase. The nature of the mechanisms controlling the change in proliferative activity is not known, but again, because of the similarity of samples obtained from various marrow sites, the agent would most likely be systemic and humoral, rather than local environmental determinants. In many respects this system resembles the changes in growth rates observed in broth-grown bacteria or in cells grown in tissue culture with increasing population density.

Another indication of control mechanisms is the equilibrium achieved between resting and proliferating compartments. Some method of maintaining this rather sophisticated interchange of cells must exist, because, for periods as long as 9 days, the balance can be maintained (Saunders and Mauer, 1969). Leukemic cells have even been found to respond to some normal control mechanism because in some patients a normal circadian pattern of mitotic activity is found (Saunders, *et al.*, 1967).

The mechanisms for control of leukemic cell growth are of obvious importance. From studies of a computer-simulated model of leukemic-cell kinetics, an important possible site of action is the time right after mitotic division, when the cell either returns promptly to the mitotic cycle for another division or goes into a resting phase (Mayer, *et al.*, In press). The metabolic events at that time in the cell are not well understood in relationship to the important decision in the cell's life. Another factor determining overall population-growth rates is cell death, a factor well appreciated in solid tumors but on which no information is available for leukemias.

An exciting and useful tool for treatment of acute leukemia has come from the observation that leukemic-cell proliferative activity can be perturbed by chemotherapeutic manipulation (Lampkin, *et al.*, 1971). With the drug effects of some agents are associated secondary effects on the population-control mechanics such that resting cells can be recruited into proliferative activity. Similar recruitment has been reported with extra-corporeal irradiation of blood leukemic cells (Chan and Hayhoe, 1971), but has not been possible by reducing the blood-leukemic-cell concentration with exchange transfusion (Lampkin, *et al.*, 1971) or leukapheresis (Reich, *et al.*, 1971). Because of the relative resistance of resting cells to cycle-dependent chemotherapeutic agents, the possibility of goading these cells into a drug-sensitive state presents a tantalizing opportunity. There is much of potential value to be learned from further studies of those control mechanisms regulating leukemic-cell growth.

Recently a new tool for study for growth regulation of cells in acute myeloblastic leukemia has become available. By means of *in vitro* culture methods, the influence of normal growth factors for myeloid cells can be assessed in malignant cell populations. These studies are already making evident that some leukemic cells respond to these normal factors by altering their growth and maturation patterns. Further definition of the relationship of normal myeloid growth factors to the leukemic cell in acute myeloblastic leukemia is awaited with obvious interest. Similar techniques applied to acute lymphoblastic leukemia would be an important advance in our study of that disease.

REFERENCES CITED

- Astaldi, G., and C. Mauri.** 1971. Recherches sur l'active proliferative de l'hémocytoblaste de la leucémie aigue. *Rev. Belg. Path. et med. exper.* 23: 69-82.
- Chan, B. W. B., and F. G. J. Hayhoe.** 1971. Changes in proliferative activity of marrow leukemic cells during and after extracorporeal irradiation of blood. *Blood* 37: 657-663.
- Cronkite, E. P., S. W. Greenhouse, G. Brecher, and V. P. Bone.** 1961. Implication of chromosome structure and replication on hazard of tritiated thymidine and the interpretation of data on cell proliferation. *Nature (London)* 189: 153-154.
- Cronkite, E. P.** 1967. Kinetics of leukemic cell proliferation. *Semin. Hematol.* 4: 415-421.
- Ernst, P., and S. A. Killmann.** 1970. Perturbation of generation cycle of human leukemic blast cells by cytostatic therapy in vivo: Effect of corticosteroids. *Blood* 36: 689-696.
- Foadi, M. D., E. H. Cooper, and R. M. Hardisty.** 1967. DNA synthesis and DNA content of leukocytes in acute leukemia. *Nature (London)* 216: 134-136.
- Gavosto, F., A. Pileri, C. Bachi, and L. Pegoraro.** 1964. Proliferation and maturation defect in acute leukaemia cells. *Nature (London)* 203: 92-94.
- Gavosto, F., A. Pileri, V. Gabutti, and P. Masera.** 1967. Non-self-maintaining kinetics of proliferating blasts in human acute leukemia. *Nature (London)* 216: 188-189.
- Hughes, W. L.** 1970. The metabolic stability of deoxyribonucleic acid. p. 83-96 *In* Stohlman, Jr., F., ed. *The Kinetics of Cellular Proliferation.* Grune & Stratton, New York. 333 p.
- Japa, P.** 1942. A study of the mitotic activity of normal human bone marrow. *Brit. J. Exp. Pathol.* 23: 272-276.
- Killmann, S. A.** 1968. Acute leukemia: The kinetics of leukemic blast cells in man. An analytical review. *Ser. Haematol.* 2: 38-102.
- Killmann, S. A.** 1965. Proliferative activity of blast cells in leukemia and myelofibrosis. Morphological differences between proliferating and nonproliferating blast cells. *Acta Med. Scand.* 178: 263-280.
- Lampkin, B. C., T. Nagao, and A. M. Mauer.** 1971. Synchronization and recruitment in acute leukemia. *J. Clin. Invest.* 50: 2204-2214.
- Mauer, A. M., C. F. Evert, Jr., B. C. Lampkin, and N. B. McWilliams.** In press. Cell kinetics in human acute lymphoblastic leukemia: Computer simulation with discrete modeling techniques. *Blood.*
- Mauer, A. M., and V. Fisher.** 1962. Comparison of the proliferative capacity of acute leukemia cells in bone marrow and blood. *Nature (London)* 193: 1085-1086.
- Mauer, A. M., and V. Fisher.** 1966. Characteristics of cell proliferation in four patients with untreated acute leukemia. *Blood* 28: 428-445.
- Mauer, A. M., B. C. Lampkin, N. B. McWilliams, and C. F. Evert, Jr.** In press. Cell kinetic patterns in human leukemia. Evidence for control mechanisms. *In* Proceedings of the Vth International Symposium on Comparative Leukemia Research, Padova-Venice, Italy, 1972. *Bibl Haematol.*
- Pileri, A., V. Gabutti, P. Masera, and F. Gavosto.** 1967. Proliferative activity of the cells of acute leukemia in relapse and in steady state. *Acta Haematol.* 38: 193-199.
- Reich, L., K. Ohara, P. Stoerzinger, and B. Clarkson.** 1971. Effect of massive leukapheresis on proliferation in acute myeloblastic leukemia (AML). (Astr.) *Proc. Amer. Assoc. Cancer Res.* 12: 25.
- Rubini, J. R., E. P. Cronkite, V. P. Bone, and T. M. Flidner.** 1960. The metabolism and fate of tritiated thymidine in man. *J. Clin. Invest.* 39: 909-918.
- Saunders, E. F., B. C. Lampkin, and A. M. Mauer.** 1967. Variation of proliferative activity in leukemia cell populations of patients with acute leukemia. *J. Clin. Invest.* 46: 1356-1363.
- Saunders, E. P., and A. M. Mauer.** 1969. Reentry of nondividing leukemic cells into proliferative phase in acute childhood leukemia. *J. Clin. Invest.* 48: 1299-1305.
- Wagner, H. P., H. Cottier, and E. P. Cronkite.** 1972. Variability of proliferative patterns in acute lymphoid leukemia of children. *Blood* 39: 176-186.

The Ecology of Animals. *N. P. Naumov*, translated by *F. K. Plous, Jr.* The University of Illinois Press, Urbana. 1972. x+650 p. \$17.50.

This textbook was first published in 1963 for use in university-level ecology courses in Russia. As such it provides an introduction to the flora, fauna, and ecology of the Soviet Union, a biota that is unfamiliar to most ecologists. Much of this information is contained in the nearly 350 tables and figures that illustrate the text. Nearly all of the analyses are graphic in nature and much of the quantitative detail of the studies is lost.

More importantly, this book is an historical document reflecting the philosophical basis for the study of ecology within the Soviet Union in the early 1960's. In an attempt to rectify ecology with Marxist doctrines, it embodies the then-accepted views of Lysenko, which denied any relationship between genetics and evolution, and which emphasized the inheritance of acquired characters. It is also concerned with practical aspects of ecology, particularly in regard to agriculture and game management. Its discussions of the ecology of individuals, populations, and communities are often narrow, and have a curiously 19th-century air about them.

JERRY F. DOWNHOWER