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Granulocyte Colony Formation in Chronic Granulocytic Leukemia during Stable, Accelerated and Blastic Disease (40049)

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Colonies of maturing granulocytes and macrophages can be grown from the peripheral blood and bone marrow of normal human beings as well as from patients with a wide variety of hematopoietic disorders (1–4). In acute granulocytic leukemia little or no colony formation takes place from leukemic cells (3–6). A number of workers have shown, however, that in chronic granulocytic leukemia (CGL), at least in the stable phase of the disease, there are increased numbers of granulocyte colony forming cells (CFU-C) circulating in the peripheral blood and the bone marrow (3, 5, 7, 8). It has also been suggested that the number of such colony forming cells decreases in the bone marrow as the accelerated or blast phase of this disease ensues (3, 4). The data supporting this contention has, however, been scanty.

The present studies were undertaken to determine the number of CFU-C in the peripheral blood and bone marrow of patients with CGL during various phases of their disease. These studies have corroborated previous work indicating that the number of CFU-C in peripheral blood during the stable phase of the disease is high. Unlike previous studies, however, the present work has shown that in the accelerated and blast phase of CGL that colony forming cell numbers may be very high or very low, but are not consistently decreased.

Materials and methods. All patients in this study were seen at the University of Colorado Medical Center between January 1, 1970 and June 1, 1977. The diagnosis of CGL was based upon a hypercellular bone marrow, the presence of a low leukocyte alkaline phosphatase score and/or the presence of the Philadelphia chromosome in bone marrow and/or peripheral blood preparations. All of these studies were performed after written informed consent as approved by the Human Research Committee of the University of Colorado Medical Center. The definitions of various stages of the disease as used in this study are as follows:

Stable phase. Patients were placed in the stable phase if they were either off all medication or on maintenance therapy and had hematocrits above 30, platelet counts above 100,000 and white blood cell counts (WBC) less than 50,000 with less than 5% blasts and promyelocytes. Three patients whose WBC counts cycle periodically above 50,000 were included because they appeared clinically to be in the stable phase of their disease.

Accelerated phase. The criteria of Schwartz and Canellos were used to define the accelerated phase (9). Patients who exhibited a gradual acceleration in their disease with progressive anemia, thrombocytopenia, leukocytosis, splenomegaly, or thrombocytosis unresponsive to previously effective therapy were considered to be in accelerated phase. Leukocytosis with up to 20% blasts was felt consistent with accelerated phase, over 20% with blast phase.

Blast phase. The criteria for definition of blast phase were drawn from previous work by Schwartz and Canellos (9), Canellos *et al.* (10), and Karanas and Silver (11). Patients who had a rapid acceleration of their disease with leukocytosis greater than 30,000/mm^3, anemia with a hemoglobin less than 10 gm%, and thrombocytopenia with platelets less than 100,000, with an FUO with T greater than 38.5° for 5 days; or with an increasing percentage of blasts in the marrow (20–30%) were considered to be in blast crisis. Patients with leukocytosis and greater than 20% blasts in the peripheral blood were placed in the blast phase category.

Fortunately, the designation of patients into the above categories was readily apparent. No difficulty was encountered in separating those in the accelerated phase from those in the blast phase.

Preparation of peripheral blood and bone marrow cells for colony growth. The tech-

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nique used here was that of Pike and Robinson using a double layer agar system (1). The stimulus for colony formation in all of these studies was peripheral white blood cell feeder layers derived from the peripheral blood of normal human beings. These were prepared as previously described in 0.5% agar in McCoy's 5A medium with 15% fetal calf serum in a concentration of 1×10^6 cells/ml, and stored in a fully humidified CO_2 incubator at 37° for up to 14 days prior to use.

Peripheral blood from patients was collected in heparinized vacuum tubes, allowed to sediment by gravity at room temperature and the white blood cell rich plasma removed. Peripheral white blood cells were then counted and plated in at least triplicate plates at concentrations of 1×10^5 and 2×10^5 nucleated cells per ml in 1 ml overlayers in 0.3% agar.

Bone marrow was collected by aspiration of the posterior iliac crest in heparinized syringes. This was followed by sedimentation at gravity at room temperature for 1–2 hr at which time the buffy coat plasma was withdrawn, the nucleated cells counted and plated as above at concentrations of 1×10^5 and 2×10^5 cells/ml. In the present studies no attempts were made to remove colony stimulating cells from the bone marrow or peripheral blood overlays.

After preparation the plates were allowed to gel at room temperature and then incubated in a fully humidified incubator at 37° for 14–18 days at which time the colony counts were performed with the aid of a dissecting microscope. Only colonies containing 50 or more cells were counted.

Results. Figure 1 shows the number of CFU-C found in the peripheral blood of patients with CGL during the stable, accelerated, and blast phases of the disease. In the stable phase of the disease 43 determinations were made on 16 patients. The mean number of CFU-C per ml of blood was 107×10^3. This is almost twice the value found in normal human peripheral blood which in our laboratory ranges from 0 to 50×10^3 colonies per ml (2). Note particularly that the number of colony forming cells in peripheral blood has been expressed per ml of blood rather than per number of cells plated. This is important because of the extreme variability in

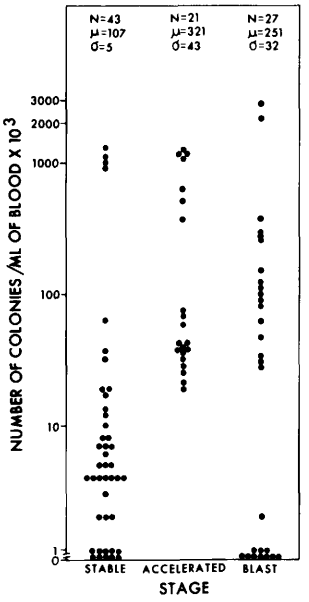

FIG. 1. The number of colony forming cells per ml of peripheral blood in patients with chronic granulocytic leukemia during the stable, accelerated, and blast phases of the disease. Each point is the mean colony count of data from one patient. The number of determinations (η), mean (μ), and median (σ) values are given.

the peripheral white blood cell numbers in these patients. In the accelerated phase of the disease, the number of colonies was further increased to 6 times the normal value with a mean colony count of 321×10^3/ml. The scatter was, however, quite broad ranging from 19×10^3/ml to 1260×10^3/ml. The data shown in Fig. 1 represents 21 data points obtained on 10 patients using the criteria for accelerated phase disease as outlined earlier. The wide scatter noted suggests that the median determination may be a more important value. This is also shown in Figure 1 for all phases of CGL and indicates that when the number found in the stable phase of the disease (5×10^3) is compared to the accelerated phase an increase (43×10^3/ml of blood) is also found. During the blast phase of the disease the number of colony forming cells was again increased over that found in normal humans and in the stable phase of CGL with a mean of 251×10^3/ml as shown in Fig. 1. The data shown here represents 27 data points on 11 separate patients. Here, once again, the scatter was quite broad. Determination of the median shown in Fig. 1,

indicated that the value was higher (32 × 10^3) than that seen in the chronic phase of the disease but similar to that found in the accelerated phase. Attempts to correlate the number of CFU-C in the peripheral blood with absolute white blood cell counts, the patients response to therapy or subsequent clinical outcome were unsuccessful. Those patients with high colony forming cell numbers in the accelerated or blast phase of the disease did not differ significantly clinically from those who had low or decreased colony forming cell numbers.

Figure 2 shows the number of CFU-C in the bone marrow of the same patients during various phases of their disease. Unfortunately, in the accelerated and blast phase of the disease bone marrow aspirates are often impossible to obtain and the number of data points available was somewhat less. In these studies the number of colonies has been expressed as per 1 × 10^5 cells plated. In the stable phase of the disease 42 data points were obtained on 22 patients. The mean number of colonies grown was 47 and the median

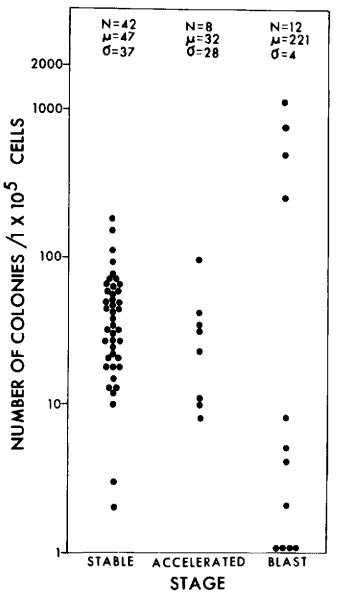

FIG. 2. The number of colony forming cells per 1 × 10^5 cells plated in the bone marrow of patients with chronic granulocytic leukemia during the stable, accelerated, and blast phases of the disease. Each point is the mean colony count of data from one patient. The number of determinations (η), mean (μ), and median (σ) values are shown.

was 37. The mean number of colonies during the stable phase of the disease is surprisingly not significantly higher than we have recently reported for normal human bone marrow grown under the same conditions (12). In those studies, 99 normal human bone marrows were plated at 2 × 10^5 cells and the mean colony forming cell number was closely clustered around 75 with a range from 24 to 250. It is unlike data reported by previous authors which indicated that the number of CFU-C in the bone marrow was markedly increased in stable CGL (3–7). Also shown in Fig. 2 are the number of CFU-C in the bone marrow of patients during the accelerated phase of the disease. Shown here are eight data points obtained on four patients. The number of CFU-C is reduced compared to normal values with a mean of 32 and a median of 28. In contrast, the mean number of CFU-C in the bone marrow of patients during the blast phase of their disease is markedly elevated at 221 colonies/10^5 cells plated. The data shown here contain 12 data points obtained on six patients. It can be seen that there was considerable scatter and when the median colony count, as opposed to the mean, is determined, this value falls to 4 CFU-C per 1 × 10^5 cells. It would appear from the data shown in Fig. 2 that in the blast phase of CGL there are two separate populations of patients, one group with high numbers of CFU-C in the marrow and another group with low numbers. No clinical differentiation between these patients could be determined, however.

There was a direct correlation between the numbers of CFU-C found in the peripheral blood and bone marrow during all phases of the disease. When high numbers of CFU-C were present in the blood, high numbers were also found in the bone marrow. The reverse was also found and is illustrated by the four patients with very low or absent bone marrow CFU-C during the blastic phase. In all four concomitant determinations of peripheral blood CFU-C also showed that they were low or absent.

Colony morphology of peripheral blood and bone marrow colonies was studied using Wright-Giemsa stained preparations. No definite differences in cellular maturation of colony cells were noted when compared between

groups or with normal human bone marrow colonies as described previously (1).

Discussion. These data have confirmed previous reports that the number of granulocyte colony forming cells in the peripheral blood of patients with chronic granulocytic leukemia is increased during the chronic or stable phase of the disease. These data have not confirmed previous reports that the number of CFU-C in the bone marrow is increased, however, nor have they shown a consistent decrease in the colony forming cell numbers in the peripheral blood or bone marrow with the advent of blast crisis, a claim which has been put forward previously (3, 4).

The reasons for the differences in colony growth noted between previous reports and those reported here do not apparently lie in the heterogeneity of patients studied. Clinical details for the patient group studied here have not been presented but they all represent so-called "classic" chronic granulocytic leukemia with the presence of the Philadelphia chromosome and/or low leukocyte alkaline phosphatase scores. Likewise, their clinical outcome with relationship to longevity or response to therapy cannot be equated with the various colony forming cell numbers noted.

It is likely that these results represent the biologic variability that might be expected in such a disease and that this represents the first large study specifically related to this disorder.

Perhaps of interest to the present studies has been the recent finding of the presence of terminal nucleotidal transferase on the cells undergoing blast crisis in chronic granulocytic leukemia (13). This data has suggested that there may be progressive lymphocyte involvement in this disorder with the final development of acute lymphoblastic leukemia. Previous studies from this laboratory have shown that the number of CFU-C in bone marrow of children with acute lymphoblastic leukemia is markedly reduced compared to normal humans (14). It would be of extreme interest to determine whether those patients in the present studies who had reduced numbers of CFU-C in the peripheral blood and bone marrow during the advanced stages of their disease were also those who appear to transform to a lymphocytic form of leukemia. These studies have not been performed to date but are currently being considered.

We originally hoped that determination of colony forming cell numbers in peripheral blood and bone marrow might be of prognostic importance and significance. The initial suggestion that as blast crisis ensued the number of colony forming cells decreased led us to consider the possibility that we might use this indicator as a guide to therapy, particularly early intervention with more aggressive chemotherapy before histologic evidence of blast crisis occurred. The present studies indicate that such an approach is neither feasible nor warranted. Patients with clear-cut blast crisis retain large numbers of colony forming cells in their peripheral blood and bone marrow. In our hands this appears to occur in about half the patients. It is possible that with serial points on a single patient one might be able to postulate the eventual outcome of the disease but this is probably not more beneficial than simple histologic viewing of changes in the morphology of the peripheral blood and bone marrow cells.

The present findings, when taken in the light of other recent studies in CGL suggest a considerable biologic and pathophysiologic spectrum in this seemingly uniform disorder of human beings.

Summary. The number of granulocyte colony forming cells (CFU-C) has been determined in the peripheral blood and bone marrow of patients with chronic granulocytic leukemia (CGL) during the stable, accelerated and blastic phases of the disease. These studies have corroborated previous work indicating that blood CFU-C numbers are high during the stable phase of CGL. In the accelerated and blast phases of CGL, CFU-C numbers in the peripheral blood and bone marrow may be decreased, normal or increased, but are not consistently low. Changes in CFU-C numbers could not be correlated with clinical patterns of CGL, or eventual outcome of the disease.

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1. Pike, B., and Robinson, W. A., J. Cell. Physiol. **76,** 77 (1970).
2. Kurnick, J. E., and Robinson, W. A., Blood **37,** 136 (1971).
3. Moore, M. A. S., Williams, N., and Metcalf, D., J. Nat. Cancer Inst. **50,** 603 (1973).
4. Sultan, C., Marquet, M., and Joffroy, Y., Nouv. Rev. Fr. Hematol. **15,** 161 (1975).
5. Craddock, C. G., Hays, E. F., Forsen, N. R., and Rodensky, D., Blood **42,** 711 (1973).
6. Moore, M. A. S., Spitzer, G., Williams, N., Metcalf, D., and Buckley, J., Blood **44,** 1 (1974).
7. Olofsson, T., and Olsson, I., Blood **48,** 351, (1976).
8. Moore, M., and Robinson, W. A., Proc. Soc. Exp. Biol. Med. **146,** 499 (1974).
9. Schwartz, J. H., and Canellos, G. P., Blood **46,** 11 (1975).
10. Canellos, G. P., DeVita, V. T., Whang-Peng, J., and Carbone, P. P., Blood **38,** 671 (1971).
11. Karanas, A., and Silver, R. T., Blood **32,** 445 (1968).
12. Entringer, M. A., Robinson, W. A., and Kurnick, J. E., Exp. Hematol. **5,** 125 (1977).
13. McCaffrey, R., Harrison, T. A., Parkman, R., and Baltimore, D., N. Eng. J. Med. **292,** 775 (1975).
14. Mangalik, A., Robinson, W. A., and Holton, C. P., Blut **34,** 77 (1977).

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