Discordant Maturation as the Primary Biological Defect in Chronic Myelogenous Leukemia

Annabel Strife, Caryl Lambek, David Wisniewski, Maria Wachter, Subhash C. Gulati, and Bayard D. Clarkson


ABSTRACT

Comparative studies of the in vitro growth characteristics of normal and chronic myelogenous leukemic (CML) progenitor cells have provided further evidence that discordant maturation is the primary biological defect in CML. The in vitro growth of total normal and CML granulocyte/macrophage colony forming unit (CFU-GM) populations were compared with early and intermediate (HLA-DR positive) CFU-GM derived from the same marrow. The absolute number of total CML CFU-GM exceeded the number generated by normal marrow through 7 days of culture due entirely to an excess of CML CFU-GM with limited proliferative capacity. Unlike normal colonies, relatively few of the leukemic colonies grew to a large size; the early and intermediate (HLA-DR positive) CML progenitors also exhibited limited proliferative capacity compared to normal. Highly enriched progenitor populations were prepared, and it was observed that the primitive (small) CML CFU-GM also had greatly reduced proliferative potential compared to primitive normal progenitors, but rather behaved similarly to normal mature (large) CFU-GM. Similarly, CML erythroid burst forming units were at a more advanced stage of maturation than normal erythroid burst forming units as evidenced by their reduced proliferative capacity, the observation that a reduced proportion required burst promoting activity to enable them to respond to erythropoietin and the observation that a larger fraction than normal could sustain a limited period of erythropoietin deprivation in the absence of burst promoting activity. Based on these findings and supporting evidence from our previous studies and those reported by other investigators, it is concluded that the dominance of the leukemic population is not due to unregulated proliferation but rather to discordant maturation resulting in expansion in the later maturation compartments which are not under strict regulatory control.

INTRODUCTION

CML has a consistent cytogenetic abnormality, the Philadelphia chromosome (Ph') (1, 2) and is known to have a clonal origin in a pluripotent stem cell which gives rise to granulocytes, erythrocytes, megakaryocytes, monocyte/macrophage cells (3), and in some patients, lymphoid cells (4). The chronic phase leukemic cells retain the capacity for differentiation and maturation, and it has been established from a study of atomic bomb survivors (5) that the average time between the original mutational event in the first stem cell and the development of clinical symptoms is 8 years. At the time of diagnosis, the Ph' positive chromosome is found in 85-100% of the proliferating marrow cells in almost all patients (6-8). Therefore, although the Ph' positive leukemic cells eventually replace the normal clone, a considerable period of time is required for this to occur.

Although the Ph' positive stem cell can generate cells of several lineages, the chronic phase of CML is typically associated with an increased production of granulocytes, and in about 50% of the patients, an increased production of platelets. We reviewed the marrow differentials of 25 newly diagnosed, untreated CML patients and found a consistent shift to higher percentages of the most mature granulocytes, bands, and polymorphonuclear cells, relative to normal marrows (46 versus 21%, respectively), resulting in an increased granulocyte maturation index in CML marrow. Anemia is often present in the chronic phase and the involvement of the erythroid lineage is less obvious, probably as a result of the difference in the dynamics of RBC production. The involvement of the lymphoid lineages during chronic phase is not consistent, most likely due to preexisting long-lived normal lymphoid clones.

The Ph' chromosome is an abnormal chromosome 22 which results from a reciprocal translocation between the long arms of chromosome 9 and 22, t(9;22) (q34;q11) (9). The c-abl oncogene located on chromosome 9q34 is translocated (10, 11) into a specific breakpoint cluster region (bcr) on chromosome 22 (12, 13). The translocation results in a chimeric bcr-c-abl transcript of 8.5 kilobases (14) which encodes a phosphoprotein P210 abl (15). P210 abl and P160 abl have similar tyrosine kinase activities distinct from the normal c-abl product, P145 abl (16), and since it has been shown that P160 abl tyrosine-specific kinase activity is necessary for cellular transformation (17), it has been suggested that the P210 abl tyrosine kinase activity may be essential in the pathogenesis of CML (18).

on the molecular level but also to define the abnormalities in the biological behavior of the Ph' positive population as precisely as possible so that the findings may be integrated to obtain a full understanding of the pathogenesis of CML. We have previously reported studies comparing the behavior of normal and CML hematopoietic progenitor cells which suggested that accelerated maturation may be the primary biological abnormality of the Ph' positive clone (19, 20). In this paper we report the results of additional studies which support the hypothesis of discordant maturation in CML.

MATERIALS AND METHODS

Human Bone Marrow Specimens. After appropriate human protection committee (Institutional Review Board) validation and informed consent, posterior iliac crest bone marrow aspirations were obtained from normal volunteers and CML patients in chronic phase. Nine CML patients were studied: one of 9 had 95% and 8 of 9 had 100% Ph positive metaphases in the bone marrow on direct cytogenetic analysis, and no additional chromosomal abnormalities were found. Buffy coat cells were collected after centrifugation of the undiluted bone marrow aspirates at 800 rpm for 10 min at 5°C and suspended in McCoy's medium supplemented with 10% FCS (Sterile Systems, Inc., Logan, UT). The diluted buffy coat cells were first depleted of platelets by centrifugation over Percoll (1.050 g/cm³) (Pharmacia Fine Chemicals Co., Piscataway, NJ), and light-density cells were subsequently obtained by centrifugation over Percoll (1.075 g/cm³) (21).

Received 8/10/87; revised 10/30/87; accepted 11/11/87.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
DISCORDANT MATURATION IN CML

Monoclonal Antibodies. Ascites fluids containing Mabs anti-B1 (22), anti-B4 (23), anti-My4 (24), anti-My8 (25), and anti-903 IgG1 form of MoI (26) were kindly supplied by Drs. James D. Griffin and Stuart F. Schlossman (Dana Farber Cancer Center, Boston, MA). Anti-human LyT-1 (27) and LyT-3 (28) Mabs were kindly supplied by Dr. John A. Hansen (Fred Hutchinson Cancer Center, Seattle, WA). Ascites fluid containing anti-glycoprotein A (R10) Mab (29) was kindly supplied by Dr. Paul Edwards (Ludwig Institute for Cancer Research, Surrey, England). Anti-Leu-1 Mab in the form of tissue culture supernatant of the cloned line (30) was supplied by Dr. Robert L. Evans (Sloan-Kettering Institute for Cancer Research, New York, NY). WEM-G11 Mab (31) was kindly supplied by Dr. Angel Lopez (Institute of Medical and Veterinary Science, Adelaide, South Australia). These Mabs are directed against antigens expressed by B- and T-lymphocytes, monocytes, granulocytes, and maturing erythroid and natural killer cells. Anti-12 Mab, which detects a nonpolymporphic region of the human IA-like antigen (32), was kindly supplied by Dr. James D. Griffin (Dana Farber Cancer Center, Boston, MA).

Enrichment of Bone Marrow Progenitor Cells by Negative Selection. Affinity plates for immunoadherence were prepared by the indirect method of Barker et al. (33) as previously described in detail (34). Light density bone marrow cells were incubated with saturating amounts of a panel of Mabs for 30 min on ice. After coating, the cells were washed twice with ice cold McCoy's 5A medium supplemented with 10% FCS and the antibody-coated cells were then depleted by immunoadherence as previously described (34).

Cell Separation by FACS IV. For these studies, bone marrow progenitor cells were partially enriched using the following panel of Mabs: anti-B1; LyT3; R10; My4; and My8 followed by immunoadherence. The cells were then incubated with anti-12 Mab for 30 min on ice. After washing, the antibody-coated cells were stained with fluorescein isothiocyanate-conjugated F(ab′)2 fragments of goat anti-mouse IgG (Cappel, Malvern, PA), washed twice, and the DR+ cells were obtained by sterile sorting using a fluorescence-activated cell sorter (FACS IV) (Becton-Dickinson, Mountain View, CA) (35). The DR+ populations were examined using a fluorescent microscope and the mean percentage positive was >90%.

Velocity Sedimentation in an Isokinetic Gradient. Highly enriched bone marrow progenitor cells (panel of Mabs: anti-B1; B4; LyT-1; LyT-3; Leu1; N901; My4; My8; 903; WEM-G11; and R10 followed by immunoadherence) were separated on the basis of cell size by velocity sedimentation in an isokinetic gradient of Ficoll as previously described in detail (19, 36). Fractions 11–12 contained progenitor cells intermediate in size, and the antibody-coated cells were partially enriched using the following panel of Mabs: anti-B1; LyT3; Leu1; N901; My4; My8; 903; WEM-G11; and R10 followed by immunoadherence. Fraction 11–12 contained progenitor cells intermediate in size, and Fractions 13–15 contained the largest progenitor cells. The most primitive progenitors reside primarily in the small cell fraction and the most mature progenitors reside primarily in the largest cell fraction (19).

Progenitor Cell Assays. To assay for CFU-GM in the earlier study of light density and DR+ bone marrow cells, the populations were cloned in 1.3% methylcellulose in McCoy's 5A medium (modified) supplemented with 20% FCS over an underlayer of 0.5% agar supplemented with 20% FCS and 20% of a selected lot of MoCM (37) which was kindly provided by Dr. David W. Golde (UCSC for the Health Sciences, Los Angeles, CA). The light density cells were cloned at 10,000–15,000 cells/ml and the DR+ cells were cloned at 200–1,200 cells/ml. Quadruplicate cultures were incubated in a humidified atmosphere of 5% CO2 in air. CFU-GM derived colonies were scored at 3, 7, 14, and 21 days.

In the later studies, the target cell populations of highly enriched bone marrow progenitor cells were cloned as previously described (36). Cell cultures contained 1 ml of IMDM (GIBCO, Grand Island, NY) containing 24% FCS, 0.8% deionized bovine serum albumin (Sigma Chemical, St. Louis, MO), 10-4 mol/liter of 2-mercaptoethanol (Sigma Chemical) and methylcellulose at a final concentration of 1.3% in 35-mm Lux tissue culture dishes (Miles Scientific, Naperville, IL). When BFU-E and CFU-GEM were assayed, 1 unit of partially purified human urinary EPO (specific activity, 49 units/mg) (Toyobo, New York, NY) was added either at the initiation of culture or 48 h later, and the cultures contained bovine hemin (ferric chloride protoporphyrin 1X) at a final concentration of 0.2 mmol/liter.

Positive control cultures for CFU-GM, BFU-E, and CFU-GEM contained 10% of a selected lot of MoCM. IMDM was substituted for MoCM in negative control cultures. In cultures stimulated by recombinant growth factors, the final concentrations of rGM-CSF and rhG-CSF were 100 pmol/liter and 500 units/ml, respectively (36).

Small (primitive) progenitor populations were cloned at 300–1500 cells/ml and intermediate and large (mature) progenitor populations were cloned at 600–5000 cells/ml. CFU-GM were scored at 3, 7, and 14 days and BFU-E and CFU-GEM were scored at 14 days using a Leitz Diavert microscope. Quadruplicate cultures were incubated in a humidified atmosphere of 5% CO2 in air.

Purified Growth Factors. Purified biosynthetic rGM-CSF was prepared as described (38, 39) and kindly provided by Drs. David Golde and Judith Gasson. rhG-CSF (40, 41) was kindly provided by Dr. Lawrence M. Souza, Amgen, Thousand Oaks, CA.

RESULTS

Total CFU-GM Populations. Light density cells (density < 1.075 g/cm3) from 5 normal and 5 CML marrows were cloned to evaluate the total CFU-GM populations. To determine if there was a significant loss of CFU-GM in the population of cells with a density > 1.075 g/cm3, this population was also cloned from 2 normal and 3 CML marrows. A mean of >95% of the CFU-GM from both CML and normal marrow was recovered in the light density fraction.

We have found that generally CML marrows have a higher percentage of light density maturing granulocytes than do normal marrows which results in a higher recovery of marrow nucleated cells in the light density fraction. In this study, the mean recovery of total nucleated cells in the light density fraction from normal and CML marrows was 31 and 54%, respectively. Since this difference affects the overall cloning efficiencies obtained (due to a greater dilution by noncolony forming mature cells in CML marrow), and since essentially all the CFU-GM were recovered in the light density fraction, we calculated for each marrow the absolute number of CFU-GM/106 nucleated bone marrow cells. With these values, we could more accurately compare the differences in absolute number of CFU-GM in normal and CML marrow.

In Fig. 1, the total mean absolute numbers of CML and normal CFU-GM/106 nucleated bone marrow cells are shown as a percentage of the number of CML CFU-GM at 3 days. Although CML marrow generated 3.6 and 2.7 times (P < 0.02, < 0.05) the total number of GM colonies generated by normal marrow at 3 and 7 days, respectively, at 14 and 21 days the
total number of GM colonies generated was the same as normal marrow. The number of CML GM colonies, therefore, fell more sharply with time than normal GM colonies. The major difference, however, was in the absolute number of GM colonies > 40 and > 50 cells. In spite of generating 3–4 times the normal number of total GM colonies, CML marrow generated only a mean of 33 and 23% of the normal number of GM colonies > 40 and > 50 cells, respectively (P < 0.001, < 0.002). The proliferative capacity, therefore, of CML CFU-GM was considerably less than that of normal CFU-GM.

We have found selected lots of MoCM to be more potent sources of CSF than leukocyte feeder layers or other crude conditioned medium we have examined (e.g., phytohemagglutinin-leukocyte conditioned medium, giant cell tumor conditioned medium, 5637), stimulating greater numbers and considerably larger GM colonies. As a result, MoCM amplifies the increased proliferative potential of normal CFU-GM relative to CML CFU-GM.

DR+ CFU-GM Populations. DR+ cells were obtained by FACS IV sorting of partially enriched populations of marrow progenitor cells derived from the same CML and normal marrows shown in Fig. 1. The mean differentials of the normal and CML DR+ populations were blasts 54 and 56%, promyelocytes 28 and 23%, and myelocytes 11 and 7%, respectively. The DR+ populations were cloned at the same time and in the same culture conditions as the light density cells.

In Fig. 2, the mean absolute numbers of DR+ CML and normal CFU-GM/10⁶ nucleated bone marrow cells are shown as a percentage of the number of CML DR+ CFU-GM at 3 days. Similar to the CML total CFU-GM population, CML DR+ CFU-GM had very limited proliferative capacity, relative to normal, generating only 20 and 16% of the normal number of GM colonies >40 and >50 cells, respectively (P < 0.001, < 0.001). However, in contrast to the increased number of CML total CFU-GM seen at 3 and 7 days (Fig. 1), there was no increase in the total number of DR+ CFU-GM, relative to normal, at any time.

CFU-GM Derived from Highly Enriched Subpopulations of Bone Marrow Progenitor Cells. Using density separations and a panel of Mabs followed by immunoadherence, we depleted normal and CML marrows of accessory and maturing cells to obtain highly enriched marrow progenitor cell populations by negative selection. We further separated, on the basis of cell size, the enriched progenitor cell populations by velocity sedimentation in an isokinetic gradient of Ficoll. An additional 4 CML patients and 4 normal donors were included in this study.

Fig. 3 shows the pattern of development of MoCM stimulated CFU-GM derived colonies from one normal and one CML primitive (small) progenitor population and one normal mature (large) progenitor population; the results in the other 3 comparative studies were similar (it was not possible to combine these results because the differential counts in the different subjects varied). As noted in the legend to Fig. 3, the populations contained 75–78% blast cells and 10–20% promyelocytes. The differentials were similar for all three populations and are therefore appropriate for comparison. The results are shown as a percentage of the number of GM colonies present at 3 days (100%), still present at 7 and 14 days of culture, and that percentage of the three-day number that obtained a size >40 and >100 cells.

The number of GM colonies derived from normal primitive (small) progenitors increased between 3 and 7 days, and 88% of the 3-day number were still present at 14 days. In contrast, the number of GM colonies derived from CML primitive (small) progenitors steadily decreased with time and only 36% of the 3-day number were still present at 14 days. Even the number of GM colonies derived from normal mature (large) progenitors increased between 3 and 7 days and a somewhat greater percentage (55%) of the 3-day number were still present at 14 days.

In addition, there was a striking difference in the proliferative capacity of normal and CML primitive (small) progenitors; 70% of the normal GM colony number present at 3 days reached a size >100 cells, whereas only 15% of the CML GM colonies reached >100 cells. Even the normal mature (large) progenitors had a greater proliferative capacity than did the CML primitive (small) progenitors (43% of the 3-day GM colony number obtained a size >100 cells). The results further demonstrate the limited proliferative capacity of even highly enriched primitive CML progenitors.

We have found that even when low numbers of enriched progenitor cells depleted of accessory cells are cloned, a subpopulation of CFU-GM can begin to proliferate to a limited
degree in the absence of CSF (IMDM cultures) (36). This latter population can be detected in our culture system containing a high percentage (1.3%) of methyl cellulose but cannot be detected in more dilute culture conditions (0.3% agar or 0.8–1.0% methyl cellulose) due to dispersion of these colonies. The limited capability of this subpopulation of CFU-GM to begin to proliferate in the absence of CSF may be due to factors present in FCS or CSF-receptor complexes existing prior to removal of the cells from the donors (42).

Fig. 4 shows the growth of the same progenitor cell populations shown in Fig. 3 in IMDM (no CSF) cultures, and in cultures stimulated by rGM-CSF and rhG-CSF. The results are shown as a percentage of the number of GM colonies in MoCM stimulated cultures (100%).

The normal primitive (small) progenitor population contained a small subpopulation of CFU-GM (25%) evident at 7 days, which could begin to divide to a limited degree in the absence of CSF (IMDM control); rGM-CSF and rhG-CSF did not increase either the size or number of these latter GM colonies. In contrast, the majority of CML primitive (small) GM progenitors could begin to divide to a limited degree in the absence of CSF (70% at 7 days), and these CML primitive progenitors responded to rGM-CSF and rhG-CSF. The normal mature (large) GM progenitors behaved very similarly to the primitive CML progenitors. Fifty-five % of the normal mature progenitors (at 7 days) could begin to divide in the absence of CSF, and these normal mature (large) GM progenitors also responded to rGM-CSF and rhG-CSF. The fact that the CML primitive GM progenitors responded better to rGM-CSF and the normal mature GM progenitors responded better to rhG-CSF is most likely only a reflection of the composition of the individual progenitor populations (neutrophilic versus eosinophilic) since rhG-CSF does not stimulate eosinophilic colonies (36).

BFU-E and CFU-GEM Derived from Highly Enriched Subpopulations of Bone Marrow Progenitor Cells. The growth of BFU-E derived from the same 4 CML and normal highly enriched progenitor cell populations was also studied. In these and previous studies, we have consistently observed that CML 14-day BFU-E derived colonies are significantly smaller than normal BFU-E derived colonies regardless of the marrow target population or whether the cells are cultured in the absence or presence of a source of BPA. Therefore, CML BFU-E exhibit the same limited proliferative capacity as do CML CFU-GM. We have found selected lots of MoCM to be more potent sources of BPA than other crude conditioned media examined (e.g., phytohemagglutinin-leukocyte conditioned medium, 5637), stimulating greater numbers and considerably larger BFU-E derived colonies. As a result, MoCM amplifies the increased proliferative potential of normal BFU-E relative to CML BFU-E.

To determine if a higher percentage of CML BFU-E relative to normal could respond to EPO without a source of BPA, we compared the number of BFU-E in cultures without a source of BPA (IMDM cultures) to the number of BFU-E in cultures stimulated by MoCM. Fig. 5 shows the growth in IMDM cultures of BFU-E derived from small, intermediate, and large progenitor cell populations when EPO was added initially. The results are shown as a percentage of the number in MoCM stimulated cultures (100%). As we had previously found (36), the number of normal BFU-E which could respond to EPO initially, without a source of BPA, increased with increasing size of the progenitor cell (mean: Fractions 4–10 48% versus Fractions 13–15 119% of MoCM). There was only a minimal difference, however, in the number of BPA independent CML BFU-E with increasing size of the progenitor cells (mean: Fractions 4–10 93% versus Fractions 13–15 119% of MoCM). The fact that in some instances the mature (larger) BFU-E gave rise to the same number or increased numbers in the absence of BPA is probably due to differentiating factors present in MoCM which caused premature terminal differentiation and dispersion of the mature erythroid colonies.

The most striking difference between normal and CML BFU-E, however, was observed in the IMDM cultures when EPO addition was delayed for 48 h (Fig. 6). The number of normal and CML BFU-E as a percentage of the number in MoCM stimulated cultures was 4–7 versus 44–63%, respectively, (P < 0.001).

A comparison of CML and normal CFU-GEM is hampered by the limited number of these hematopoietic progenitor cells. However, we have observed that CML CFU-GEM are generally greatly reduced in size, relative to normal CFU-GEM. This limited proliferative potential of CML CFU-GEM is consistent with the limited proliferative potential of CML CFU-GM and BFU-E. The frequency of CFU-GEM in subpopulations of highly enriched marrow progenitor cells appeared to be less than normal, but this may not be statistically significant.

DISCUSSION

To fully understand the pathogenesis of CML, it will be necessary to precisely define the abnormalities in the biological
behavior of the Ph¹ positive clone and integrate the biological findings with the precise abnormalities on the molecular level. In an attempt to determine the consistent biological abnormalities of the neoplastic clone, we have compared several different normal and CML hematopoietic target populations. In this report, we have compared the growth characteristics of (a) total CFU-GM populations derived from light density marrow cells with DR+ CFU-GM populations derived from the same marrows, (b) primitive normal and CML CFU-GM with mature normal CFU-GM derived from highly enriched marrow progenitor cell populations, and (c) BFU-E and CFU-GEM derived from highly enriched subpopulations of bone marrow progenitors.

Since essentially all the marrow CFU-GM were recovered in the light density fraction, the absolute number of CFU-GM/10⁶ nucleated bone marrow cells could be calculated for each CML and normal marrow. The absolute number of CML CFU-GM was about 3-fold greater than normal marrow through 7 days of culture. The increase was entirely due to CFU-GM with very limited proliferative capacity, since the absolute number of CML GM colonies >50 cells was only 23% of the absolute number generated by normal marrow. There was no increase observed in the absolute number of CML DR+ CFU-GM derived from the same marrows and in addition, the CML DR+ CFU-GM exhibited the same limited proliferative capacity, generating only 16% of the normal number of GM colonies >50 cells. The cells having a proliferative potential which were excluded from the DR+ populations were more mature DR negative promyelocytes and essentially all of the myelocyte population. This suggests that an increase in the number of mature promyelocytes and myelocytes having limited proliferative capacity is largely responsible for the granulocyte expansion in CML. Even with the increase in marrow cellularity in these CML patients (a mean of 4.4 times the normal marrow cellularity in these studies), the absolute number of CML CFU-GM with greater proliferative potential per milliliter of bone marrow did not exceed the normal number. This further suggests that mature CFU-GM with limited proliferative potential are responsible for the increased production of granulocytes in CML.

Highly enriched marrow progenitor cell populations derived from normal and CML marrow were separated on the basis of cell size by velocity sedimentation. As we have previously reported (19, 36), we and other investigators have found that progenitor cells differing in size also differ in their degree of maturation, with the most primitive progenitors residing primarily in the small cell fractions and the most mature progenitors residing primarily in the largest cell fractions.

To compare the growth characteristics of normal and CML subpopulations of CFU-GM, we cloned the cells in the absence of CSF and in cultures stimulated by MoCM, rGM-CSF, and rhG-CSF. We have recently reported (36) that rGM-CSF and rhG-CSF primarily affect the growth of relatively mature subpopulations of normal CFU-GM. In the same report (36), we found that even when very low numbers of highly enriched progenitors depleted of accessory cells are cloned, a subpopulation of CFU-GM can be detected which can begin to divide, to a limited degree, in the absence of CSF. The frequency and size of these GM colonies did not increase with increasing numbers of cells cloned (50-4000 cells). Although it is possible that factors in the FCS may be responsible for the initiation of proliferation, a more likely explanation may be CSF-receptor complexes existing prior to removal of the cells from the donors (42). If the latter is the case, one would expect to have a higher percentage of mature progenitors beginning to divide in the absence of CSF, and as we have shown, this was the case.

The growth characteristics of CML primitive (small) CFU-GM were similar to normal mature (large) CFU-GM, and both differed significantly from normal primitive (small) CFU-GM. Only a minor population of normal primitive CFU-GM could begin to divide, to a limited degree, in the absence of CSF with no additional augmentation by rGM-CSF and rhG-CSF. In contrast, the majority of CML primitive and normal mature CFU-GM could begin to divide in the absence of CSF and both responded to rGM-CSF and rhG-CSF. In addition, there was a striking difference in the proliferative capacity of normal and CML primitive (small) progenitors; 70 versus 15%, respectively, of the GM colony number present at 3 days attained a size >100 cells. Even the normal mature (large) progenitors had a greater proliferative capacity than did the CML primitive (small) progenitors (43% of the 3-day GM colony number obtained a size >100 cells). The results suggest that the growth characteristics of the CML primitive CFU-GM are not unique but rather resemble the growth characteristics of normal mature CFU-GM.

We have consistently observed that CML BFU-E are significantly smaller than normal BFU-E and therefore exhibit the same limited proliferative capacity of CML CFU-GM. Although we have not seen any consistent difference in the frequency of BFU-E in CML marrow compared to normal marrow, the reduced proliferative capacity of CML BFU-E would
result in a reduction of RBC production and could explain the anemia often present in CML patients.

In a previous study (36), we examined the effect of delaying the addition of EPO on the population of BFU-E, immediately dependent on EPO for proliferation and terminal differentiation but not requiring an exogenous source of BPA. As in this previous study (36), the number of normal BFU-E which could respond to EPO in the absence of BPA, when EPO was added at the initiation of culture, increased with increasing size of the progenitor cells. However, there was less distinction between CML primitive and mature BFU-E, due to a higher percentage of BPA independent CML primitive BFU-E. The striking difference between normal and CML BFU-E, however, was seen when EPO addition was delayed for 48 h. The number of normal BFU-E which survived in the absence of BPA was reduced to 4–7% of the number in MoCM stimulated cultures in contrast to 44–63% of CML BFU-E. The results suggest that in normal marrow, there is a minor population of BFU-E which no longer requires BPA for survival or stimulation and has EPO-receptor complexes sufficient to sustain the erythroid progenitor for a limited period of EPO deprivation and that this subpopulation of BFU-E is significantly expanded in CML marrow. Therefore, a greater proportion of CML marrow BFU-E appear to be at a more advanced stage of maturation than in the normal marrow BFU-E population.

A comparison of normal and CML CFU-GEM is hampered by the limited number of these progenitor cells. However, we did observe that CML CFU-GEM were generally greatly reduced in size, relative to normal CFU-GEM, which is consistent with the limited proliferative capacity observed for CML CFU-GM and BFU-E.

We first reported evidence suggesting accelerated maturation concomitant with a reduction in proliferative capacity of Ph¹ positive progenitor cells in a study of light density marrow cells separated by velocity sedimentation (19). In the same report, we referred to our earlier study of core bone marrow biopsies (7) and results reported by other investigators which provided further evidence for accelerated maturation in CML. We referred to the biological abnormality as “accelerated” maturation because the primitive Ph¹ positive progenitors appeared to be entering the later maturation compartments more rapidly than normal. However, this term may be misleading since their passage through the later maturation compartments does not appear to be accelerated and in fact their total transit time through the maturation compartments may be longer than normal because of an increased number of divisions and a longer life span. As will be discussed, we believe discordant maturation describes the abnormality more accurately.

The cytokinetics of CML has been extensively reviewed (20) and the evidence is that CML marrow granulocyte precursors have lower mitotic indices and [³H]thymidine LI than comparable normal marrow granulocyte precursors (43). Stryckmans et al. (44) found that the [³H]thymidine LI of CML myeloblasts increased to near normal values when the WBC of the CML patients was <20,000/mm³ but was lower than normal when the WBC was >40,000/mm³. In addition, they found that the [³H]thymidine LI of CML myelocytes was unaffected by the WBC. They concluded that the proliferation of CML myeloblasts is sensitive to the size of the myeloid cell mass and that CML myeloblast proliferation is controlled by one of the regulatory mechanisms controlling normal granulopoiesis. They suggested that the absence of such a regulatory control acting on CML myelocytes is a reflection of the later maturation stage of myelocytes and the same may be true for normal myelocytes. The cytokinetic evidence would therefore indicate that the proliferation of immature CML granulocyte precursors is subject to regulation by normal growth control mechanisms.

There have been studies reported indicating that the maturation of Ph¹ positive granulocytes and megakaryocytes is asynchronous. In an electron microscopic study by Asano and Kawahara (45), it was shown that the cytoplasmic maturation of CML promyelocytes was more rapid than the nuclear maturation. Pedersen (46) reviewed several abnormalities of CML polymorphonuclear granulocytes concluding that they were related to neutrophil maturation, and that in CML, there is peripheral circulation of many incompletely mature neutrophils. The incomplete maturation of a proportion of morphologically mature granulocytes is most likely responsible for their increased life span. In another electron microscopic study by Vainchenker et al. (47), it was found that the cytoplasmic maturation of CML megakaryocytes in culture was also more rapid than the nuclear maturation.

Recently, we have extensively reviewed the reported abnormalities of Ph¹ positive leukemic cells from our own studies and studies by other investigators, and from the summation of the evidence, proposed a hypothesis for the primary biological defect in CML (48). We concluded that the primary biological abnormality in CML which results in an increased mass of myeloid cells is not unregulated proliferation of the Ph¹ positive leukemic cells but rather discordant maturation. Proliferation of the more primitive leukemic progenitors could still be subject to normal growth regulatory control mechanisms, but with asynchronous maturation and a greater proportion entering into the maturation compartments, the Ph¹ positive leukemic population could expand mainly in later maturational compartments (e.g., late blasts, promyelocytes, and myelocytes) not subject to growth regulatory control. The more rapid cytoplasmic maturation, cited earlier, may be responsible for the progenitors entering the later maturational compartments, and the retarded nuclear maturation may endow the more mature leukemic progenitors with the capability of undergoing more divisions and having a longer life span than the comparable normal mature progenitors. This hypothesis would predict that a considerable period of time would be required before the Ph¹ positive leukemic population could outnumber the normal clone as was indeed found to be the case in the study of atomic bomb survivors (5).
Discordant Maturation as the Primary Biological Defect in Chronic Myelogenous Leukemia

Annabel Strife, Caryl Lambek, David Wisniewski, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/48/4/1035

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.