CHRONIC GRANULOCYTIC LEUKEMIA: THE DEVASTATING CONSEQUENCES OF GENETIC INSTABILITY

MEDICAL GRAND ROUNDS

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1. INTRODUCTION

Chronic granulocytic leukemia (CGL) is a clonal hematopoietic neoplasm of unknown etiology characterized by a neutrophilic leukocytosis with a left shift. The leukemic cells from at least 90% of patients contain a characteristic reciprocal chromosome translocation t(9;22); the 22q-chromosome is called the Philadelphia (Ph') chromosome after the city of its discovery. This differentiated tumor of the bone marrow inevitably undergoes a metamorphosis characterized by progressive loss of myeloid cell maturation, poor response to treatment, and short survival. Dr. Frenkel last reviewed this disease here 10 years ago (1). Since that time, important new insights into pathogenesis have been gained that will be emphasized in this review. Unfortunately, these advances in understanding have not yet resulted in increased survival for the majority of patients. However, it has been established that allogeneic bone marrow transplantation for younger patients in the chronic phase of CGL has the potential to cure this disease, and these results will be reviewed as well. My bias in this presentation is that a primary defect in CGL is an acquired tendency to genetic instability of multipotent hematopoietic stem cells which gain a selective growth advantage over normal precursors. Other hypotheses are perhaps equally tenable, since there is no proof that the signs of genetic instability observed in CGL cells are primary in the pathogenesis of the disease. These changes could still be remote secondary effects of some underlying derangement that is as yet not understood.

Clinical, morphologic, and cytogenetic observations suggest that CGL follows a stepwise evolution which can be divided into three phases:

1) the initial, presymptomatic stage
2) the chronic stage
3) the metamorphosis--variously called the accelerated, transformed, blastic, or terminal stage by different authors.

Considerable variation is manifest in the order of development of clinical, morphologic, and cytogenetic features during these different phases. Therefore, conclusions on the biologic significance of cytogenetic changes cannot be made with certainty.

2. INITIAL PHASE OF THE DISEASE

2.1 Etiology and epidemiology

The etiology of CGL is not known. It is clear that exposure to ionizing radiation increases the risk of the disease in a dose-dependent fashion. This effect was seen in the atom bomb survivors. Most of the leukemias seen in heavily exposed individuals were acute and chronic granulocytic (2-5). The peak of excess incidence of leukemia was noted 7 years after exposure, but the risk for individuals who received the greatest exposure was still above background 14 years after the bombing. In British patients with ankylosing spondylitis who were treated with radiotherapy, almost all the resulting leukemias were chronic myelogenous (6-8). The fact that ionizing radiation can increase the risk of CML is consistent with the hypothesis that a maladaptive response to genetic damage, perhaps resulting in persistent genetic instability, can initiate CGL. In the vast majority of cases, however, there is no evidence of exposure to radiation.

Exposure to benzene and alkylating agents increases the risk of acute granulocytic leukemia. In contrast, there is little evidence that these agents induce CGL.

Few clues to the causation of CGL have emerged from epidemiologic or familial studies (9,10). CGL accounts for 15-20% of all leukemias in Western countries, and the mortality rate is about 1 per 100,000 per year. Most of the patients are between 25 and 60 years old, with the peak incidence between 40 and 49 years. In contrast to children with acute leukemia, the concordance rate for CGL in identical twins is not high. However, the overall rarity of the disease makes it difficult to exclude the significance of heritable factors in the etiology of CGL.

After marrow ablative therapy and allogeneic bone marrow transplantation, CGL frequently recurs. Most of these recurrences have been in host, but a few have been in donor blood cells (11). These donor recurrences suggest that an ongoing leukemogenic process is at work in these patients. The nature of this process is unknown.

2.2 Evidence for the existence of a stage of CGL prior to the establishment of the Ph' chromosome

Recent clinical and cytogenetic evidence suggests that CGL may begin as a clonal expansion of hematopoietic cells which do not contain the Ph' chromosome. Lisker et al described two patients with clinical and hematologic features of CGL whose leukemic cells acquired the Ph' chromosome only after a 6-13 week period of observation (12). Fialkow et al have used the expression of X-linked glucose-6-phosphate dehydrogenase (G-6-PD) alleles as clonal markers of various hematopoietic lineages to gain insight into the natural history of the CGL clone (13). In individual adult hematopoietic stem cells and their progeny, only one of these two X-linked loci is expressed. The other is permanently inactivated early during embryogenesis, before ontogeny of the hematopoietic system. Since the selection of the allele for inactivation is random in each individual cell, in a heterozygous individual with normal polyclonal hematopoiesis both alleles will be expressed. The progeny of any one stem cell will produce only one allele. This form of the enzyme, therefore, becomes a clonal marker for descendents of that stem cell. The establishment of this clonal marker antedates formation of the Ph' chromosome, since the latter is found only in hematopoietic cells; whereas specific X chromosome inactivation characterizes every somatic cell in the body. Therefore, this marker has become extremely useful in establishing not only the extent of clonal involvement of various hematopoietic lineages in CGL but also the presence of the Ph' chromosome in these CGL lineages as well. The studies are done in black women, since they are the only readily available subjects who are commonly heterozygous for the G-6-PD marker. The two forms of the enzyme found in these subjects are named A and B, and these forms can be distinguished from each other by differential migration on starch gel electrophoresis.

Early experiments with CGL patients who were G-6-PD heterozygotes showed that granulocytes, monocytes, eosinophils, erythrocytes, and platelets all expressed the same single isoenzyme. This confirmed that CGL was a clonal disorder of multipotential stem cells (14). Subsequently, in an especially revealing study, Martin et al examined a G-6-PD heterozygote whose CGL cells expressed only the B form of the enzyme (15,16). One issue addressed by this study was

the possibility that some T and B lymphocytes were also derived from the neoplastic stem cell (15). After immortalization of blood lymphocytes *in vitro* with the Epstein-Barr virus, 74 B lymphocyte cell lines were obtained for analysis (Table 1).

TABLE 1. KARYOTYPE AND G-6-PD PHENOTYPE OF B LYMPHOCYTE LINES FROM A CGL PATIENT

	G-6-PD Phenotype	
Karyotype	A	B
Ph+	0	9
Normal	14	25
Ph'-Aneuploid	0	8

G-6-PD Phenotype of CGL: B
Ratio A:B = 18:45 (Ph'-) ($p < 0.001$, x^2)

(See references 15,16.)

Nine of these cell lines contained the Ph' chromosome while 65 did not. This provided cytogenetic evidence that at least some of the B lymphocytes obtained from this patient were derived from the CGL clone. Of the 63 Ph' negative lines analyzed, 45 (71%) expressed only the B form of G-6-PD. This fraction was higher than would be expected had these lines originated only from normal, polyclonal B lymphocytes and, therefore, suggested that at least some of these Ph' negative B lymphocytes had originated from the CGL stem cell. Furthermore, of 33 Ph' negative, G-6-PD type B enzyme containing cell lines that were adequately karyotyped, 8 were chromosomally abnormal. By comparison, none of 14 lines that expressed only the A type of G-6-PD had any cytogenetic abnormalities. Thus, B lymphocytes were identified which shared the same clonal G-6-PD isoenzyme marker with the CGL granulocytes and which contained a variety of non-Ph' chromosomal abnormalities. These results are consistent with the hypothesis that a genetically unstable, Ph' negative hematopoietic stem cell is established early in the natural history of CGL. According to this hypothesis, both the non-Ph' cytogenetic changes and the Ph' translocation develop in descendents of this cell. The Ph' chromosome may confer a proliferative advantage specifically upon granulocyte precursors which are responsible for the massive granulocytic hyperplasia which characterizes the chronic phase of the disease. Later, other cytogenetic abnormalities arise that contribute to the progressively more malignant behavior of the leukemia during metamorphosis.

The findings could also be explained by assuming that the Ph' translocation is the event that initiates the leukemia and that, subsequently, both the 22q- and 9q+ chromosomes are lost from certain progeny. This scenario seems very unlikely, since both alleles of chromosome 9 and 22 are present and normal in appearance in all 8 of the cytogenetically abnormal lymphoid cell lines (16).

2.3 Evidence against a stage of CGL that antedates the development of the Ph' chromosome

At least as many clinically and hematologically normal people whose blood cells are Ph' positive have been reported as have been patients whose blood cells acquired this abnormality only some period after developing the chronic phase of CGL. Individuals found to be Ph' have been followed and have later developed typical CGL (17). In a surveillance study of 41 radiation-related patients, the Ph' chromosome was uniformly present in bone marrow cells at the time when a leukocytosis was first noted (18). The study was not designed to detect cytogenetic abnormalities in subjects prior to the development of hematologic abnormalities.

2.4 Summary-pathogenesis of the first phase

The evidence strongly suggests that CGL may be established as a clonal hematopoietic expansion prior to the establishment of the Ph' chromosome. Critical cytogenetic observations of presymptomatic patients are understandably very scant, and the presence of the Ph' chromosome in these patients does not exclude the possibility of such a pre-Ph' phase. From the available data, we can conclude that by the time most patients exhibit significant leukocytosis, the Ph' translocation has already developed. However, the Ph' chromosome does not appear to be an absolute prerequisite for granulocytic hyperplasia in every case. Here is one example of the lack of a tight correlation between clinical, hematological, and cytogenetic events in CGL. Because of this heterogeneity, firm conclusions about the biologic function of the Ph' chromosome cannot be made. One rare opportunity to dissect the early events in the induction of CGL may found in the patient who has received a bone marrow transplant and whose disease recurs in donor hematopoietic cells (11).

2.5 Clinical features of prodromal CGL

The Japanese study cited above (18) plus other observations suggest that CGL may evolve in a rather indolent fashion without symptoms for a period of months to a few years. The order of events gleaned from serial observations of radiation-exposed patients is shown in Figure 1.

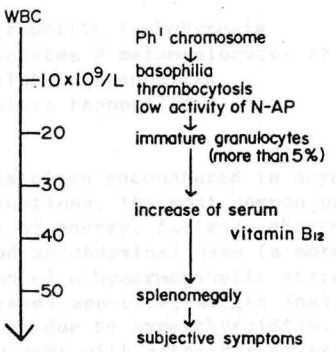

FIGURE 1. Chronological sequence in appearance of abnormalities characteristic of CML.

(See reference 18.)

The Ph' chromosome was present when the white count was around 10,000. Symptoms arose when the white count was about 70,000/µl. Occasionally, such a presymptomatic patient will be detected by routine screening, usually with a white count under 100,000/µl. Other than confirmation of the diagnosis by bone marrow aspiration and cytogenetic examination, no therapy is recommended for this stage of disease.

3. THE CHRONIC, SYMPTOMATIC PHASE OF CGL

3.1 Clinical and hematologic features

This is the phase of the disease that is most familiar to internists, who have the opportunity to make this diagnosis from a clinical evaluation of the patient and examination of the peripheral blood smear. The signs, symptoms, and laboratory profile of these patients have been summarized by Spiers (19,20) (Table 2).

TABLE 2A. CGL: CHRONIC PHASE SYMPTOMS

Loss of energy
Fatigue
Change in appetite
Weight loss
Abdominal mass or discomfort
Sweats, heat intolerance

TABLE 2B. CGL: CHRONIC PHASE SIGNS

Splenomegaly (90%)
Pallor, tachycardia
Sternal tenderness

TABLE 2C. CGL: CHRONIC PHASE HEMATOLOGY

Neutrophilic leukocytosis
Myelocytes > metamyelocytes >> blasts
Absolute eosinophilia
Absolute basophilia

Although the disease is often encountered in asymptomatic patients during routine screening evaluations, the most common presenting symptoms (in order of frequency) are loss of energy, fatigue, shortness of breath, pallor, anorexia, weight loss, and an abdominal mass (a more common complaint in women). Less commonly, symptoms of a hypermetabolic state are noted: night sweats, heat intolerance,increased appetite, weight loss, and tremor. These symptoms can be misinterpreted as due to hyperthyroidism. Uncommon modes of presentation include secondary gout with arthritis or nephrolithiasis, angina or claudication, sudden left upper quadrant pain from splenic infarction or rupture, and symptoms of leukostasis such as visual disturbances, deafness, vertigo, or priapism.

Presenting physical signs may be totally absent. Signs of anemia may be noted, but infection and purpura are uncommon. Splenomegaly is a cardinal sign of the disease and is found in about 90% of cases. The size of the spleen may range from a mere tip to massive enlargement that can be missed due to failure to locate the inferior pole. In the latter case, it is best to palpate for the right edge of the spleen by moving the examining hand from right to left, starting to the right of the umbilicus. CGL and myelofibrosis are the most common causes of massive splenomegaly in this and other Western countries. A lesser degree of hepatomegaly may be present. Lymphadenopathy is unusual in the chronic phase and, if found in the presence of CGL, is suggestive of metamorphosis. Tenderness over the lower sternum is a frequent finding. Rarely, hyperleukocytosis may lead to sausaging of the retinal veins, papilledema, deafness, or priapism.

Laboratory investigation is necessary to establish the diagnosis. Spiers has stressed the importance of the differential count which is dominated by neutrophils (bands plus segmented forms) and myelocytes, which together account for a mean of 75% of all white cells. There are fewer metamyelocytes than myelocytes and very few (1-2%) myeloblasts and promyelocytes. Elevated absolute basophil and eosinophil counts are seen in virtually every case, even though these cells usually account for <5% of the differential. Thus, absolute basophil and eosinophil counts that are within the normal range raise some doubt about the diagnosis of chronic phase CGL. The hemoglobin is usually normal or moderately decreased; it is rarely increased. The platelet count is often moderately to markedly elevated, but occasionally is decreased. The neutrophil leukocyte alkaline phosphatase is usually, but not always, decreased, and elevated levels of serum vitamin B_{12} binding protein reflect the release of transcobolamin I from the expanded mass of granulocytes. Bone marrow aspiration and biopsy is done to confirm the diagnosis. This usually reveals pronounced hypercellularity with granulocytic hyperplasia, but may also contain a moderate amount of fibrosis. The most important reason for doing a bone marrow examination is to obtain material for cytogenetic analysis. Most of the metaphases from at least 90% of patients who fit the clinical and hematologic profile described above will contain the Ph' (22q-) chromosome, generally as the sole abnormality. In most cases, normal metaphases are absent or present as a small minority of the total (21). These findings attest to the proliferative advantage of granulocyte precursors which have inherited this chromosome (Table 3).

TABLE 3. NUMBER OF CML PATIENTS IN EACH GROUP

	Status	Number of Patients	Percentage
Ph¹ negative	Negative	18	10.1%
	Negative with other chromosomal abnormalities	3	1.7%
Ph¹ positive	Positive	116	64.8%
	Positive with negative	10	5.6%
	Positive with other chromosomal abnormalities	24	13.4%
	Positive turned to other chromosomal abnormalities	8	4.4%
Total		179	100%

(See reference 21.)

3.2 Evidence for clonal expansion of a multipotential stem cell in chronic phase CGL

Shortly after its discovery in the metaphases of most marrow cells from patients with CGL, the Ph' chromosome was shown to be present in erythroid and megakaryocyte as well as granulocyte precursors (22). It was not found in mitogen-stimulated blood lymphocytes or skin or marrow fibroblasts. These findings were interpreted as evidence for a clonal expansion of a neoplastic pluripotent stem cell in this disease. Fialkow pointed out that the widespread distribution of the Ph' chromosome was not necessarily consistent with this interpretation, since an etiologic agent could transform multiple stem cells and induce this translocation in every affected cell. His studies of expression of G-6-PD isoenzymes in heterozygotes with CGL (referred to previously) established that erythrocytes, granulocytes, monocytes, and platelets were clonally derived since all of these cells derived from any single heterozygote expressed the same enzyme phenotype (14). These observations conclusively demonstrated that at the time of sampling during chronic phase, the myeloid side of hematopoiesis was clonally derived in CGL. Skin and marrow fibroblasts expressed both enzyme types, suggesting that the clonal expansion was restricted to marrow hematopoietic cells. As mentioned above, Fialkow's group subsequently assayed T and B lymphocytes cultured from G-6-PD heterozygotes with CGL. The findings suggest that at least some B lymphocytes are members of the CGL clone, whereas others are not. The situation with T lymphocytes is more complex. In patients whose disease is well controlled, T cells express a mixture of enzyme phenotypes consistent with non-clonal derivation. However, in patients with poorly controlled CGL, the distribution of enzyme phenotypes in T cells is skewed to the type expressed by the CGL clone (23). This suggests that under these circumstances, as with B lymphocytes, at least some T cells are derived from the neoplastic stem cell. The mixture of clonal and non-clonal B and T lymphocytes in the presence of an overwhelming majority of clonal myeloid hematopoiesis was interpreted to indicate that some long-lived lymphocytes which differentiated from stem cells prior to the neoplastic transformation persisted into the chronic phase of the disease. Another possibility is that the Ph' chromosome does not confer the same proliferative advantage on lymphocyte as upon granulocyte precursors.

Other evidence supports the idea that at least some B and T lymphocytes in CGL derive from the neoplastic clone. Fauser *et al* showed that T lymphocytes within multilineage hematopoietic colonies cultured from CGL patients contained the Ph' chromosome (24). Nitta *et al* cultured B and T lymphocytes from patients with CGL and prepared metaphases from these cells (25). B cell lines were obtained from 16 patients. All 448 lines derived from 12 patients (8 chronic, 2 accelerated, 2 blastic phase) were Ph' negative. Four patients (2 chronic, 1 accelerated, 1 blastic phase) gave rise to Ph' positive B cell lines. Four of 37 lines from 2 chronic phase patients were Ph' positive, while all 72 lines from an accelerated phase patient and all 53 lines from a blastic phase patient were Ph' positive. Of 461 T lymphocyte metaphases examined from 10 patients (9 chronic, 1 early blastic phase), only 1 metaphase contained the Ph' chromosome. These results again suggest that at least some B lymphocytes in CGL patients are derived from the neoplastic clone. Since the culture techniques may not support the growth of all types of lymphocytes equally, quantitative estimates of the fraction of lymphocytes that were derived from the CGL clone cannot be made. In fact, there is some evidence that Ph' positive B cell lines derived from CGL patients may have a

limited proliferative capacity in vitro (15,25). This evidence raises the interesting possibility that the product of the Ph' chromosome could be toxic for lymphocyte precursors.

3.3 Persistence of cytogenetically normal myeloid cells in CGL marrow

In most CGL patients, all metaphases derived from marrow contain the Ph' chromosome. An important question is whether normal stem cells persist in these patients. The Eaves' group has shown that cytogenetically normal myeloid progenitors emerge from long-term cultures of marrow cells derived from both untreated and treated patients with CGL (26,27). These results are interesting not only because they suggest that normal myeloid progenitors persist in CGL patients, but also that these normal progenitors may have a growth advantage under certain in vitro conditions even though the Ph' positive progenitors seem to possess a growth advantage in vivo. Clinically, some but not all patients treated with intensive chemotherapy during the chronic phase repopulate their marrows with cytogenetically normal cells (28,29). These results have led to trials of intensive chemotherapy which are designed to eradicate the Ph' positive clone. The results of these trials are summarized in Section 3.6.4. These clinical results further suggest that normal stem cells that could potentially seed a restoration of normal hematopoiesis persist in at least some CGL patients.

3.4 Molecular biology of the Ph' translocation

The brightest recent achievement in unraveling the mystery of CGL is undoubtably the molecular dissection of the Ph' translocation. In fact, we now have a nearly complete understanding of the sequences around the breakpoint on chromosome 22 and of the components of the abnormality that are constant in both classic and variant translocations. This information has suggested certain hypotheses about the biologic role of the translocation that can now be tested.

3.4.1 Cytogentic fine mapping of the Ph' defect

The Ph' chromosome was first detected as an abnormally short G group element by Nowell and Hungerford in 1960 (30). Subsequent studies by Rowley, done after banding techniques had been developed, showed that the classic translocation (found in about 90% of cases) was a reciprocal translocation between chromosome 22 and 9 with breakpoints involving band 9q34 and 22q11 (t(9;22)(q34;q11)) (31). Recent work of Prakash and Yunis using methotrexate and other synchronization methods to obtain elongated metaphase chromosomes has further refined the location of the breakpoints to bands 9q34.1 and 22q11.21 (32). The importance of these high resolution banding methods is that complex variant translocations that involve chromosomes 9, 22, and a third chromosome have been shown invariably to involve the same breakpoints on chromosomes 9 and 22 (33). The implication is that these loci are somehow important in the pathogenesis of the disease.

3.4.2 Molecular analysis of the Ph' chromosome

The steps leading to current understanding really began with experiments designed to alter the pathogenesis of Moloney murine leukemia virus. This virus regularly induces thymus-dependent lymphoma-leukemias in susceptible mice after a 4-6 month latent period. To test whether ablation of the thymus

prior to viral inoculation would alter the pathology of this tumor, Abelson treated a mouse with prednisolone before administration of the virus (34). The animal developed a bone marrow-derived lymphoma-leukemia after a latent period of only 5 weeks. From this tumor a virus was isolated which reproduced this unusual pathology in susceptible mice. Ultimately, this rapidly acting Abelson murine leukemia virus (A-MuLV) was shown to have recovered a highly conserved gene from the mouse (called c-abl) which, when modified by recombination with viral genes, was responsible for the rapid neoplastic transformation of murine hematopoietic cells in vitro as well as in vivo (35-37). The gene as modified by the virus (called v-abl) was found to encode a tyrosine specific protein kinase which in many ways is similar to the v-src gene product that had earlier been shown to be responsible for transformation of avian fibroblasts infected with Rous sarcoma virus. Genetic recombination with viral sequences at its head end seemed to unleash the kinase activity of the c-abl gene product (38). Most of the tumors induced by A-MuLV turned out to be cells arrested at a very early stage of differentiation of B lymphocytes. However, A-MuLV was also shown to be capable of transforming cells of macrophage phenotype (39) as well as increasing myelopoiesis in long-term cultures of mouse bone marrow (40).

After molecular clones of the v-abl gene became available and the precursor c-abl gene was shown to be highly conserved in the animal kingdom, studies began to exlore the possibility that this gene plays a role in human neoplasia. The chromosomal localization of the normal human c-abl gene was investigated first. Initially, Heisterkamp et al localized the gene to chromosome 9 by analysis of DNA digests isolated from human-mouse and human-human somatic cell hybrids (Table 4) (41).

TABLE 4. LOCALIZATION OF c-abl ON HUMAN CHROMOSOME 9

	Human chromosomes																					
Hybrids	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	*c-abl*
MOG-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MOG-2 E5	+	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
F4SC13C1112	+	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	+	+
MOG-2G1	+	−	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	−	+	−
SIR-7A2	+	+	−	−	+	−	+	−	−	+	+	+	−	+	+	+	+	+	+	+	+	−	+	−
SIR-7D1	+	+	−	+	−	−	+	−	−	−	−	+	+	+	+	−	+	+	+	+	+	−	+	−
SIR-7G1	+	−	−	+	−	−	+	−	−	+	+	−	+	+	−	+	+	+	+	+	+	−	+	−
HOR19D2	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	+	−
F4SC13C19	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−
MOG-13/22	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
FIR 5R3	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−
DUR 4.3	−	−	+	−	+	NT	−	−	−	+	+	+	+	+	+	−	+	+	−	+	+	+	+	−

The derivation of mouse and human somatic cell hybrids, determination of their complements of human chromosome, and identification of human *c-abl*-specific oncogene sequences was as previously described (Heisterkamp et al. 1983b).

(See reference 41.)

Only those hybrids that retained human chromosome 9 possessed the human c-abl gene as determined by hybridization with v-abl molecular probes. Since this result suggested the possibility that the c-abl locus might be involved in the Ph' translocation, Groffen's group initiated a collaboration with a team

of Dutch cytogeneticists who prepared human-rodent somatic cell hybrids derived from CGL leukocytes. Hybrids were available with the 9, 22, 9q+, and 22q- (Ph') chromosomes isolated on a rodent background, and analysis of their DNAs with abl probes demonstrated that the c-abl locus was absent from the 9q- and present on the 22q- chromosome. This was the first confirmation that the Ph' translocation is actually reciprocal, since the fraction of chromosome 9 translocated to chromosome 22 is minute and difficult to identify cytogentically. Shortly thereafter, using the technique of in situ hybridization, the same group confirmed that the c-abl locus normally resides very close to the terminus of the long arm of chromosome 9, at band 9q34 (43) (Figure 2).

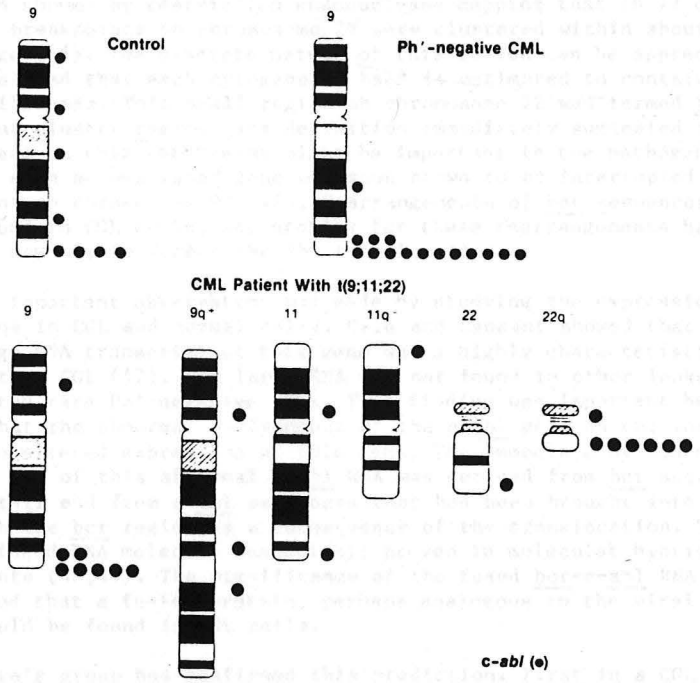

FIGURE 2. Distribution of silver grains on chromosomes hybridized to c-abl probes. A significant ($p > 0.001$) grain accumulation is noted over band q34 of chromosome 9 in a healthy control. In a CML patient with a t(9;11;22), the hybridization signal ($p < 0.001$) is found both on the normal chromosome 9 and 22q-, whereas in the Ph'-negative CML a grain accumulation is only found on chromosome 9q34 ($p < 0.01$).

(See reference 43.)

This location is near the breakpoint on the 9q+ chromosome found in the Ph' translocation (31,32). The same translocation of c-abl to 22q- was found in two complex variant translocations. This finding suggested that the critical component of both classical and variant translocations was the movement of loci on the tip of chromosome 9, including the c-abl locus, to chromosome 22.

Groffen et al next prepared molecular clones of DNA sequences located near the breakpoints of the Ph' chromosome, using probes made from the front end of the c-abl gene to identify the relevant recombinant clones (44). By "walking" away from the c-abl sequences, they were soon able to identify a clone that carried sequences derived both from chromosome 9 and 22. Using sequences derived from chromosome 22 that were very near to the breakpoint as a probe, they then showed by restriction endonuclease mapping that in 17 different CGLs the breakpoints on chromosome 22 were clustered within about a 6 kilobase range (45). The discrete nature of this region can be appreciated when it is realized that each cytogenetic band is estimated to contain 5,000-20,000 kilobases. This small region on chromosome 22 was termed bcr for breakpoint cluster region. Its definition immediately suggested that a functional gene on this chromosome might be important in the pathogenesis of CGL. In fact, such an expressed gene was soon shown to be interrupted by the breakpoint on chromosome 22 (46). Rearrangements of bcr sequences were regularly found in CGL cells, and probing for these rearrangements has become a powerful new way to detect the Ph' translocation.

The next important observation was made by studying the expression of the c-abl gene in CGL and normal cells. Gale and Canaani showed that an abnormally large RNA transcript of this gene was a highly characteristic finding in Ph' positive CGL (47). The large RNA was not found in other leukemias, including the rare Ph' negative CGLs. This finding was important because it showed that the abnormal environment of the c-abl gene on chromosome 22 resulted in altered expression of this gene. The immediate hypothesis was that the head end of this abnormal c-abl RNA was derived from bcr sequences on 22 and the tail end from c-abl sequences that had been brought into juxtaposition with the bcr region as a consequence of the translocation. The existence of this fused RNA molecule was quickly proven in molecular hybridization experiments (48,49). The significance of the fused bcr-c-abl RNA was the likelihood that a fusion protein, perhaps analogous to the viral gene product, would be found in CGL cells.

Owen Witte's group has confirmed this prediction. First in a CGL cell line and subsequently in fresh leukemia cells, Witte found by immunoprecipitation analysis that the c-abl gene product has a molecular weight of 210,000 (p210) while the size of the counterpart protein from normal cells is 145,000 (p145) (50,51). Moreover, the CGL molecule catalyzes the phosphorylation of tyrosine groups in itself as well as other proteins, whereas such kinase activity cannot be demonstrated with the normal c-abl protein. The kinase activity of the p210 molecule is remarkably similar to the function of the Abelson viral oncogene product which appears to have been activated by recombination of murine c-abl with Moloney murine leukemia viral gene segments. Both Witte et al and Canaani et al have shown that the CGL protein contains antigenic determinants that are derived from both bcr and c-abl sequences, formally proving that this is a fusion protein (52). Thus, by fundamentally different mechanisms, this oncogene has been activated by genetic recombination in both A-MuLV and in CGL cells.

The Groffen and Canaani/Gale teams have now sequenced and analyzed cDNA clones derived from CGL cells (45,46,49,52). The results indicate that the breakpoints on chromosome 22 are found either ahead of or behind the third exon of the bcr gene (Figure 3).

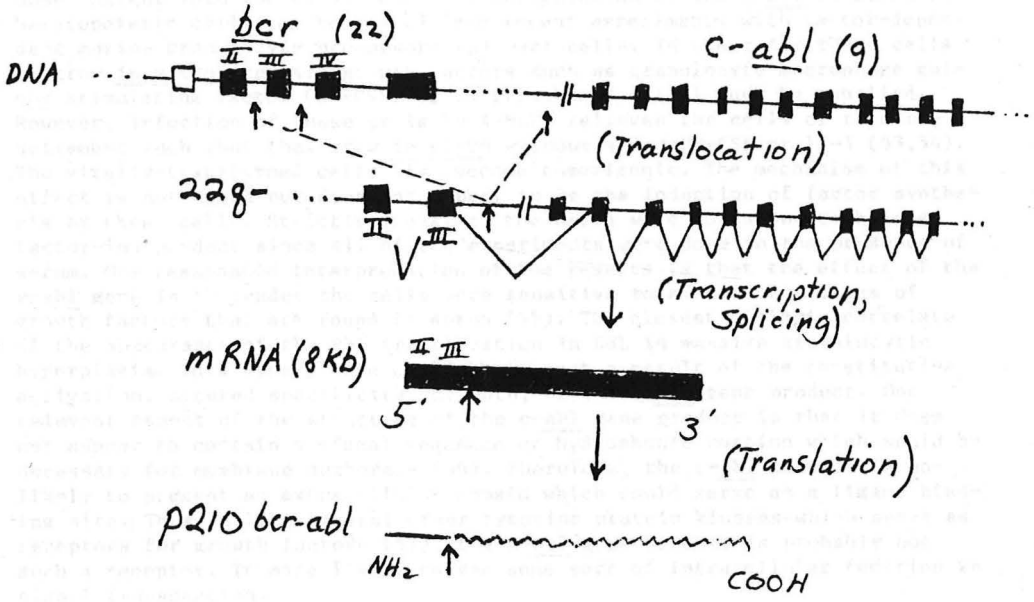

FIGURE 3. t(9;22) translocation creates a fusion bcr/abl gene.

Since the third exon is small, the difference between these two forms of the fusion protein would only amount to 25 amino acids, and it is unclear whether the two variants have different effects in CGL. The breakpoint on chromosome 9 occurs well ahead of the first known of the 11 exons of c-abl. However, the final messenger RNA is spliced in such a way that the first known c-abl exon is lost. The reason for this loss appears to be the absence of a splice acceptor site upstream of the first known c-abl exon. In one case, the RNA is spliced in such a way that the junctional codon is composed of one nucleotide from bcr sequences and two nucleotides from the second c-abl exon. Coding sequences from both bcr and c-abl genes are preserved in frame, as would be predicted from the finding of c-abl peptides at the carboxy terminus of the fusion protein.

3.4.3 Possible role of the bcr-c-abl protein in CGL

Nothing is yet known of the normal function of the bcr gene. It is reasonable to speculate that modification of the amino terminus of c-abl affects either the magnitude or specificity of function of the c-abl protein in CGL cells. Possibly the protein phosphorylation function of c-abl becomes constitutive in CGL cells. Moreover, the gene sequences upstream of c-abl that may control

its expression have been disrupted by the translocation in a way that now places the expression of a truncated c-abl gene under the control of the bcr promoter and regulatory regions.

Some insight into the consequences of deregulation of the c-abl function for hematopoietic cells can be gained from recent experiments with factor-dependent murine granulocyte precursors and mast cells. In order for these cells to grow in vitro, certain growth factors such as granulocyte-macrophage colony stimulating factor (GM-CSF) or interleukin 3 (Il-3) must be supplied. However, infection of these cells by A-MuLV relieves the cells of this requirement such that they grow in vitro without added GM-CSF or Il-3 (53,54). The virally-transformed cells also become tumorigenic. The mechanism of this effect is not known but does not appear to be the induction of factor synthesis by these cells. Strictly speaking, the cells were not shown to become factor-independent since all of the experiments were done in the presence of serum. One reasonable interpretation of the results is that the effect of the v-abl gene is to render the cells more sensitive to minute quantities of growth factors that are found in serum (55). The closest biologic correlate of the appearance of the Ph' translocation in CGL is massive granulocytic hyperplasia. This hyperplasia could be in part a result of the constitutive activation, altered specificity, or both, of the c-abl gene product. One relevant aspect of the structure of the c-abl gene product is that it does not appear to contain a signal sequence or hydrophobic portion which would be necessary for membrane anchorage (56). Therefore, the c-abl product is unlikely to present an extracellular domain which could serve as a ligand binding site. Thus, unlike several other tyrosine protein kinases which serve as receptors for growth factors (57), the c-abl gene product is probably not such a receptor. It more likely serves some sort of intracellular function in signal transduction.

3.5 New insights from current understanding: Ph' negative CGL and Ph' positive acute leukemias

The molecular delineation of the Ph' translocation has provided a new definition of the essential feature of this abnormality--the juxtaposition of bcr and c-abl sequences. This information is leading to a re-examination of the unusual CGLs that do not contain a 22q- chromosome. These cases are of interest because it has been thought that patients with Ph' negative CGL have a shorter survival than those with Ph' positive disease (58,59). The number of observations are still limited, but already show that some of the Ph' negative cases contain the functional equivalent of the Ph' configuration either on a cytogenetically normal chromosome 22 or on another chromosome (60). Other cases appear to be "true Ph' negatives" in the sense that they lack fused bcr-c-abl sequences (61). It will now be of interest to correlate clinical features and survival in the classic Ph', variant Ph' and Ph' negative groups redefined by molecular analysis.

Similar efforts are underway to analyze the molecular configuration of the Ph' chromosomes found in certain de novo acute leukemias. The t(9;22)(q34; q11) translocation has been found in a small fraction of acute lymphoblastic and acute granulocytic leukemias which begin without any suggestion of a preceding hematologic disorder (62,63). At issue is whether these cases represent CGL presenting without any chronic phase or whether these represent distinct entities. Prognostically, these patients respond poorly to treatment, just as do patients in the blastic phase of CGL (62-65). However, other

clinical features suggest that some of the de novo cases differ from blastic phase CGL in certain important respects (62-66). These are unusual patients and only a few cases have been studied to date. Prakash and Yunis have shown that the cytogentic breakpoints in two patients with Ph'+ ALL and two with Ph'+ AGL were in the same bands as those found in 20 patients with typical CGL (32). Hagemaijer et al demonstrated that the c-abl locus was translocated to chromosome 22 in one patient with Ph' positive ALL (67). Further studies which correlate molecular configuration with clinical features are needed.

3.6 Management of the chronic phase of CGL

3.6.1 Chemotherapy

The goal of therapy for most patients in the chronic phase of CGL is to control symptoms by suppressing the drive to overproduce myeloid cells (20,68). When the white count exceeds 500,000/μl, treatment is urgently needed to minimize the risks of irreversible organ system damage due to leukostasis. After initiation of allopurinol and volume repletion, the white count can be rapidly lowered with cytosine arabinoside 100 mg/m^2 every 12 hours, cyclophosphamide 1,000 mg/m^2 once a day, or hydroxyurea 1 gm/m^2 every 8 hours, repeated for 2 or 3 days as necessary. Leukaphoresis may also be temporarily effective while awaiting the onset of action of chemotherapy. In most newly diagnosed patients, such emergency therapy will not be necessary but should be given unhesitatingly if organ dysfunction is thought to be due to leukostasis.

For routine palliative treatment, there is a trend away from the old standby of CGL therapy, the alkylating agent busulfan (20). This trend has gained strength with the increasing awareness of the potential of busulfan for severe long-term side effects. The principal toxicity is a severe and occasionally irreversible pancytopenia, but a chronic wasting syndrome and pulmonary fibrosis are less common, long-recognized liabilities. The great advantage of busulfan is its simplicity of use and low cost. Control of granulocytosis and thrombocytosis is reasonably smooth and long-lasting. The initial dose need not exceed 2.5 mg/m^2/day. The drug must be tapered and stopped as the white count falls, before the normal range is reached, in order to minimize the risk of pancytopenia. The white count often continues to fall after treatment is discontinued. Therapy can often be stopped for many weeks.

The ribonucleotide reductase inhibitor hydroxyurea has become the treatment of choice for the chronic phase of CGL in many centers, primarily for reasons of drug safety (20). The myelosuppressive effects begin rapidly and are rapidly reversible. Therefore, overdosages are rarely a serious problem. The kinetics of activity unfortunately create the need for frequent monitoring of the blood counts; this and the high cost of the drug represent its major disadvantages. Continuous therapy is usually needed to control the disease. The initial dose is about 3 g/day, and the maintenance dose is usually in the range of 0.5 to 2.0 g/day.

Several interesting problems can arise in therapy. First, the platelet count, which is usually somewhat more difficult to reduce than the white count, can begin to fall towards subnormal levels on doses of therapy required to control the granulocyte mass. This phenomenon may signal the beginning of the metamorphosis (see below). In patients treated with busulfan, switching to

cyclophosphamide can overcome this problem (1). Second, a large spleen can cause abdominal discomfort. The spleen usually shrinks in proportion to the falling white count and rarely presents major problems in the chronic phase. Third, the white count of the occasional patient will spontaneously cycle between wide extremes (69,70). If this phenomenon is not recognized, drug therapy may be inappropriately administered.

3.6.2 Radiotherapy

Splenic irradiation was a popular and reasonably effective mode of therapy for CGL in the past. Radiotherapy is infrequently used nowadays in the management of the chronic phase. A large British trial concluded that the quality and duration of life in CGL patients treated with busulfan exceeded that achieved with radiotherapy (71). Splenic irradiation is occasionally useful in controlling the disease but must be given in tiny fractions--for example, 10 Rads/day to a total dose of 250 Rads--to minimize the risk of pancytopenia.

3.6.3 Splenectomy

Since the spleen is often a source of morbidity in CGL and is thought to represent the initial site of metamorphosis in some patients, the idea that early splenectomy might prolong the course of the disease was proposed long ago. Splenectomy in the poorly controlled patient may have disastrous consequences including bleeding and postoperative thrombosis. Once this was recognized, trials of splenectomy were undertaken after the disease was controlled with chemotherapy. Earlier uncontrolled trials and a recently published controlled trial clearly show that survival is not prolonged by early splenectomy (72). Many have suggested that the quality of life after metamorphosis may be improved by splenectomy, since massively enlarged spleens are often refractory to therapy during this phase. However, the quality of life after metamorphosis was not improved by early splenectomy in the British randomized trial (72). Currently, splenectomy should not be a routine procedure but should be performed on individual cases only when the anticipated benefits outweigh the risks. Splenic rupture is obviously one such indication.

3.6.4 Intensive chemotherapy and splenectomy

In these experimental trials the hypothesis was that eradication of the Ph' clone during the chronic phase might delay the onset of metamorphosis or even cure the disease. It is now clear from the results of the L5 and L15 trials at Memorial-Sloan Kettering (73,74) and of the ROAP 10 trial at M.D. Anderson Hospital (75) that this approach prolongs survival only marginally at best (76). The problem seems to be the lack of selectivity of existing drug regimens for the leukemic clone as opposed to persisting normal clones. A marginal survival advantage was seen in the minority of patients whose Ph' clone was markedly suppressed, but the differences in survival were more likely to be due to selection of prognostically different groups than to effects of therapy (73-76). When better drugs are developed, these trials will need to be repeated.

3.6.5 Effects of interferon

Partially purified leukocyte interferon alpha (9-15 x 106 units/day IM) clearly has antiproliferative effects in CGL. Useful responses including modest shrinkage of the spleen were obtained in 5 of 7 previously untreated or minimally treated patients (77). At least 50% regressions in severe symptomatic thrombocythemia were obtained in 8 of 9 patients in patients refractory to standard chemotherapy (78). Therapy was complicated by mild to severe flu-like side effects. Clearly, more trials with interferon are warranted. An important question is whether interferon exerts differential effects on normal and leukemic progenitors. A return of at least some normal bone marrow metaphases was seen in 5 of 7 patients during treatment with interferon (77).

3.6.6 Allogeneic bone marrow transplantation--a bold attempt to eradicate the malignant clone

At present, allogeneic bone marrow transplantation following ablative therapy with cyclophosphamide and total body irradiation in chronic phase patients appears to be the only therapy with curative potential in CGL (Figure 4).

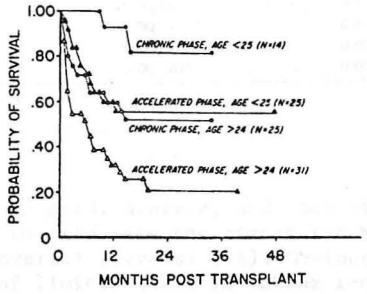

FIGURE 4. Life-table analysis showing probability of survival for younger and older patients transplanted in chronic phase or in accelerated phase of chronic myelogenous leukaemia.

(See reference 80.)

Earlier studies of identical twin transplants indicated that the actuarial chances of complete remission and survival at 5 years each to be about 60% (79). This procedure clearly induces cytogenetic remissions in the majority of patients. Results of allogeneic transplants compiled by the International Bone Marrow Transplant Registry (80) have recently been updated (81). In a group of 199 patients transplanted in the chronic phase, the actuarial chance of survival at three years is 53%. Almost all of the deaths are attributable to complications of the transplant procedure such as early infections, interstitial pneumonia, or graft-versus-host disease. The encouraging statistic is that the actuarial risk of relapse at three years is only 11%. In other words, the complete remissions obtained are often quite durable and, if transplant complications can be avoided, survival is likely to be prolonged. Unfortunately, because of lethal complications of transplantation, this therapy is feasible only for the small minority of patients who are young (under

age 45) and who have HLA matched sibling donors. Survival in patients under the age of 25 is clearly better than in older patients (80,81). The dilemma is that the one year mortality from lethal transplant complications of infection, interstitial pneumonia, and graft-versus-host disease is about 35%, and many patients who are treated according to traditional methods will survive at least 5 years in reasonably good health. Attempts to come to grips with this problem have focused upon the definition of risk groups in CGL. There is some evidence that the risk factors listed in Table 5 are associated with early metamorphosis and short survival (82,83).

TABLE 5. PROGNOSTIC SIGNIFICANCE OF DISEASE FEATURES REGULARLY REPORTED AMONG 678 PATIENTS DIAGNOSED BEFORE 1978.

Feature (Direction of Worst Prognosis)	Prognostic Significance (p Value)		
	Univariate Analysis	Multivariable Regression	
		Simultaneous	Stepwise
Sex (male)	0.5	0.06	0.09
Age (higher)	0.02	0.0009	0.001
Spleen size (larger)	0.000001	0.00003	0.00005
Liver size (larger)	0.00001	0.09	0.10
Hematocrit (lower)	0.0004	0.7	0.7
WBC count (higher)	0.001	0.6	0.6
Platelet count (higher)	0.001	0.001	0.004
Percent blasts in blood (higher)	0.000001	0.00003	0.000001

(See reference 83.)

Based on assignment to good, average, and poor risk groups, some investigators have attempted to calculate the timing for bone marrow transplantation that will optimize overall survival (84). Obviously, this is a statistical adventure which is of limited value in making individual decisions. The prognostic features are of limited accuracy. In considering transplantation, it is appropriate to remember that after the first two years, the mortality rate in CGL is remarkably constant at 25% per annum (85).

Autologous bone marrow transplantation has not been evaluated in chronic phase CGL. In the absence of methods to selectively destroy leukemic stem cells in vitro, this procedure would seem to have no rationale.

4. METAMORPHOSIS: ACCELERATED AND BLASTIC PHASES

4.1 Clinical features

Sooner or later, typically after 3-4 years of good health in chronic phase, something bad happens to almost every patient with CGL. The gravity of this change is emphasized by the fact that the median survival from this point is a matter of months. The bewildering aspect of this change is the heterogeneity of clinical and hematological features (19,20,86) (Table 6).

TABLE 5. SUMMARY OF HAEMATOLOGICAL CHANGES WHICH MAY BE OBSERVED IN CHRONIC GRANULOCYTIC LEUKAEMIA DURING METAMORPHOSIS[a]

Erythropoietic cells	
Haemoglobin	anaemia (common), polycythaemia (rare)
Erythrocyte morphology	tear-drop and pencil forms, hypochromia, anisocytosis, poikilocytosis
Reticulocytes	decreased or absent (common), increased (rare) circulating normoblasts, occasionally bizarre normoblasts suggestive of erythroleukaemia
Bone marrow	erythropoietic aplasia (rare), megaloblastoid features, frank erythroleukaemia (uncommon), sideroblastic change
Platelet series	
Platelet count	elevated (1000 to 4000 × 10^9/l), depressed (commoner)
Platelet morphology	giant platelets, abnormal granulation, megakaryocyte fragments
Platelet function	abnormal adhesion and aggregation
Bone marrow	megakarocytes reduced or absent (common), megakaryocytes increased, changes of megakaryocytic leukaemia with giant forms and small megakaryoblasts (rare)
Granulocytic series	
Neutrophils	progressive reduction (common), elevation (generally transient), defective lobation, agranular forms
Neutrophil function	neutrophil alkaline phosphatase sometimes elevated, myeloperoxidase-negative forms, defective phagocytosis
Basophils	often elevated initially, later disappear
Eosinophils	sometimes elevated initially, later disappear, forms with eosinophil and basophil granules (rare)
Myelocytes	usually decreased (leukaemic hiatus), agranular forms, monocytoid forms
Monocytes	bizarre monocytoid blast cells, may be lysozyme-positive (uncommon)
Blasts	range from absent to extreme elevation (300 to 600 × 10^9/l), bizarre forms including apparent 'lymphoblasts', Auer rods (very rare)
Bone marrow	granulocytic:erythroid ratio increased (usual), or reduced (rare), maturation arrest, blasts range from 5 to 95% but usually over 30%, isolated blast cell islets may be seen on trephine specimen
General	
Marrow fibrous tissue	usually slightly increased, extreme fibrosis with collagen formation and bone resorption may occur
Blood coagulation	disseminated intravascular coagulation with hypofibrinogenaemia may occur

[a]This list is not exhaustive. Changes associated with splenectomy are omitted. It is common for a patient to develop several changes in succession, e.g. leucopenia may appear and later give way to leucocytosis.

(See rererence 19.)

The only consistent feature is a failure to respond to previously effective chemotherapy. Symptoms, signs, and hematologic changes may occur in any order. A minority of patients develop a sudden generalized blastic proliferation followed by fulminant symptoms and rapid deterioration. A more common pattern is a progressive shift to less mature granulopoiesis in the marrow, increasing numbers of immature granulocyte precursors in the blood, and gradually advancing anemia and thrombocytopenia. Sometimes refractory thrombocytosis is seen. This pattern has been called the accelerated myeloproliferative phase of CGL (86). Occasionally, a picture of pancytopenia and myelofibrosis is seen. Symptoms include malaise, fatigue, depression, anorexia,

wasting, fever of unknown origin, sweats, bone pain, arthralgias, and abdominal distention or pain related to massive splenomegaly or hepatomegaly. An interesting phenomenon is the development of focal extramedullary metamorphosis which may begin almost anywhere and present with a great variety of symptoms. Masses of granulocyte precursors may proliferate as nodules in the skin, within lymph nodes, as single or multiple osteolytic bone lesions with hypercalcemia, retro-orbital lesions, breast masses, ulcerating gastrointestinal lesions, or diffusely as meningeal leukemia. The best policy when presented with an unusual symptom in a patient with CGL is to rule out nonleukemic conditions and perform a complete hematologic evaluation. Biopsy of suspicious lesions is often diagnostic.

4.2 Heterogeneity of blastic transformation

As noted, the marrow and/or other organs either suddenly or, more often, gradually are infiltrated with immature hematopoietic cells. The wide variety of phenotypes represented by these blast cells has provided further confirmation of the multipotent stem cell origin of CGL (87-92). Although myeloblastic transformation is the most common, proliferation of blasts of every hematopoietic lineage has been described. About 20% of blastic transformations phenotypically resemble non-T cell acute lymphoblastic leukemia in every respect, including the expression of terminal deoxynucleotidyl transferase (TdT), the common acute lymphoblastic leukemia surface antigen (CALLA), immunoglobulin gene rearrangements, and cytoplasmic IgM heavy chains (mu chains) (87,88). The proliferation of these B lymphocyte precursors was the earliest evidence that a lymphoid lineage could be involved in CGL. Erythroblastic (89), megakaryoblastic (90), basophilic (91), eosinophilic (92), and, more recently, T lymphoblastic transformations (93-95) have all been described. Occasionally, mixed proliferations are seen, including lymphoblastic and myeloblastic forms (88).

4.3 Cytogenetic changes in metamorphosis

Cytogenetic changes are common in the metamorphosis of CGL. In fact, the appearance of new cytogenetic abnormalities which are superimposed upon the Ph' chromosome has been shown to predict the onset of metamorphosis (96,97). The most common changes are the acquisition of a second Ph' chromosome, an isochromosome for the long arm of #17, and a chromosome 8 (33). There is no specificity of the latter two changes for CGL, as these abnormalities are seen in a variety of myeloid leukemias and myeloproliferative disorders. Many other secondary numerical and structural aberrations have been catalogued in transformed CGL. In some patients, no new changes are evident at the cytogenetic level and, rarely, the Ph' chromosome is lost. The biologic and molecular significance of these changes is not yet clear. In a general sense, the accumulation of cytogenetic changes often correlates with tumor progression in a variety of malignancies. These secondary abnormalities provide further evidence of the genetic instability that characterizes CGL. The often focal nature of blastic transformation supports the idea that clonal evolution underlies this phenomenon.

4.4 Management of the patient in metamorphosis

4.4.1 Chemotherapy

Cure of CGL in general and its metamorphosis in particular is not possible with currently available chemotherapy. The goals are strictly palliative and need to be tailored to the individual patient. For patients with rapidly progressive and disabling systemic symptoms, often the best that can be offered is transfusions, analgesics, and corticosteroids. On the other hand, for patients with the accelerated myeloproliferative syndrome a change in chemotherapy often can provide useful palliation of symptoms and control of cell proliferation for many months (19,20,86). We have been particularly successful with various combinations of hydroxyurea, cytosine arabinoside, and cyclophosphamide. For full-blown blastic transformation, phenotypic analysis of the blasts may help guide therapy. Up to 50% of patients with lymphoblastic disease respond to vincristine/prednisone therapy with complete or useful partial remissions (98,99). Maintenance of these remissions has been a problem, but therapy of the type used in acute lymphoblastic leukemia prolongs survival at a modest cost in toxicity. Myeloblastic disease rarely responds to vincristine/prednisone and, in fact, is notoriously resistant to aggressive antileukemic therapy of any kind (100). In myeloblastic crisis, survival is uncommonly prolonged with aggressive therapy, because complete remissions are rarely obtained. Clinical trials of new drugs are needed. Outside of the investigational setting, a palliative approach is recommended.

4.4.2 Bone marrow transplantation

Survival of patients in accelerated or blastic phases who receive marrow ablative therapy and allogeneic HLA matched bone marrow is shorter than that of similarly treated chronic phase patients. Actuarial survivals at 4 years of accelerated and blastic phase patients so treated were 30 and 12%, respectively (80,81). The major reason for these inferior results is an increased actuarial risk of relapse of leukemia, which is approximately 60%. Median survival of patients transplanted in metamorphosis is very brief, but, again, this is the only therapy that seems capable of providing any patients long-term freedom from disease.

An important observation derived from these trials has been that allogeneic bone marrow transplantation can reverse the myelofibrosis associated with CGL and restore a pattern of normal hematopoiesis (101). Thus, the myelofibrotic form of metamorphosis is not a contraindication to marrow transplantation.

Goldman et al conducted an interesting trial of autologous transplantation during metamorphosis. After ablative treatment cryopreserved blood cells that were harvested during the chronic phase were infused (102). The aim was to restore chronic phase hematopoiesis and abort the accelerated or blastic phases. The results of these trials were modest at best. Chronic phase hematopoiesis was briefly restored in many patients. However, metamorphosis recurred within a few months in most patients, and survival more than one year after grafting was obtained in only about 30% of the patients. No patient survived more than three years after transplantation, including those who received a second autograft.

5. SUMMARY, CONCLUSIONS, AND PROSPECTS

Chronic granulocytic leukemia has taught the hematologist much about normal and leukemic hematopoiesis. The disease points inescapably to the existence of a multipotential lymphoid-myeloid stem cell. This disease provided the first example of a non-random chromosome aberration in neoplasia. Many more have since been characterized. The significance of the Ph' chromosome is now unfolding. This rearrangement illustrates one way that growth-regulatory genes can be permanently deranged in a naturally developing neoplasm. The disease also provides a striking example of stepwise neoplastic development of clinical, morphological, and cytogenetic characteristics (103). At the basis of this progression seems to be an acquired tendency toward genetic instability which generates the tumor stem cell variants that frustrate our attempts to control the disease. The fundamental causes and mechanisms of this instability remain unknown, but must be near the center of the problem of pathogenesis. More effective and selective therapy should evolve from a clearer understanding of the function of the bcr-c-abl fusion protein. However, if the Ph' chromosome drives only the chronic phase of this leukemia, blockade of its function is unlikely to cure the disease. Thus, the need to understand the early steps in pathogenesis is critical. More effective control of chronic phase progenitors may delay emergence of metamorphosis by decreasing the likelihood of subsequent genetic changes. Finally, the progress in controlling the complications of bone marrow transplantation should eventually make this potentially curative form of therapy available to more patients with this lethal disease.

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