Congenital Marrow Failure Syndromes and Malignant Hematopoietic Transformation

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ABSTRACT

In the current era of advanced supportive care and administration of recombinant cytokines and other effective therapies, patients with congenital (inherited) marrow failure syndromes usually survive the early years of life and beyond. With the extended lifespan, a new “natural history” for these syndromes is evident. Although these disorders were always classified as “benign” historically, it is now evident that most of these conditions confer an inordinately high predisposition to myelodysplastic syndromes and acute myelogenous leukemia (MDS/AML). Since carcinogenesis occurs as a sequence of events that is driven by genetic damage and by epigenetic changes, the hypothesis is advanced that the first “hit” or leukemia-initiating step is the constitutional genetic mutation, itself, that initially manifests as a single lineage or multiple lineage marrow failure. The leukemic promotion and progression steps leading to MDS/AML can then ensue readily in the initiated pool of progenitors or stem cells. Thus, the distinction between benign and malignant hematologic in the context of the inherited marrow failure disorders has become blurred and new definitions for these syndromes should be developed. The Oncologist 1996;1:354-360

INTRODUCTION

In the context of this review, bone marrow failure is defined as decreased production of one or more of the major hematopoietic lineages on a congenital basis. The term “congenital” implies an early onset of aberrant hematology, sometimes at birth. Although the designations are used loosely, “congenital” marrow failure is not necessarily “constitutional” and may be due to acquired factors such as viruses and environmental toxins. With respect to the syndromes described herein, “inherited” is probably the best designation since the genetic patterns of inheritance are well defined for many of these conditions. Because hematopoiesis is an orderly but complex interplay of stem and progenitor cells, marrow stromal elements, and positive and negative cellular and humoral regulators, marrow failure can potentially occur at a number of critical points in the hematopoietic lineage pathways. For the inherited marrow disorders, the notion is advanced that genetic mutations interfere with hematopoiesis and account for the marrow failure, although the specific molecular basis is not yet known for any of these conditions. Acquired factors may also be operative and may interact with the putative genetic mutations to produce overt disease with varying clinical expression.

Historically, the inherited marrow failure syndromes were classified as “benign” hematology, which contrasted sharply with the malignant myeloid disorders. Patients with severe clinical forms of these disorders, such as Kostmann’s syndrome/congenital neutropenia, Shwachman-Diamond syndrome, Fanconi’s anemia, and congenital amegakaryocytic thrombocytopenia, often died early in life from complications of marrow failure, usually sepsis and/or bleeding. However, in the current era of advanced supportive care and administration of recombinant cytokines and other effective therapeutics, patients with these conditions usually survive the early years of life and beyond. With the extended lifespan of patients, a new natural history for some of these disorders is evident. One of the most sobering observations is that most of these “benign” disorders confer an inordinately high predisposition to myelodysplastic syndromes (MDS), acute myelogenous leukemia (AML), or variations of both (MDS/AML). Thus, the distinction between “benign” and
“malignant” hematolgy in the context of the inherited marrow failure disorders has become blurred, and a new clinical and hematologic continuum can be described.

**Fanconi's Anemia (FA)**

This classical marrow failure disorder is inherited in an autosomal recessive manner and is remarkable for its diversity in phenotype [1]. At presentation, patients may have: (A) typical physical anomalies but normal hematolgy; (B) normal physical features but abnormal hematolgy (marrow failure and/or MDS/leukemia), or (C) physical anomalies and hematologic changes, the so-called classic phenotype. There can be sibling heterogeneity in presentation with discordance in clinical and hematologic findings, even in affected monozygotic twins.

The basic defect in FA is unknown. Five complementation groups and hence five separate genes (termed types A, B, C, D, and E) have been distinguished on the basis of somatic cell hybridization experiments [2-4], with FA type A (FA-A) accounting for over 65% of the cases analyzed [5, 6]. A cDNA for the group C gene was identified in 1992 and localized to chromosome 9q22.3 [7]. Using the same successful expression cloning method, a cDNA representing the group A gene has just been reported [8]. Even though the precise functions of the FA-C and FA-A proteins are unclear so far, complementation analysis suggests that they operate in concert with at least three additional proteins—the products from the FA-B, FA-D, and FA-E genes. These FA proteins may thus control or participate in a conceptually novel biochemical process which must play an important role in the various physiologic and cellular processes implicated in the Fanconi phenotype such as skeletal development, blood-cell formation, and maintenance of genomic integrity. Cloning the remaining genes should help to eventually understand this process at the molecular level.

The two theories of pathogenesis of FA relate to either defective DNA repair or an inability of Fanconi cells to remove oxygen-free radicals that damage cells [1]. The confirming laboratory test is the abnormal chromosome pattern seen in metaphase preparations of phytohemagglutinin-stimulated peripheral blood lymphocytes. These reveal breaks, gaps, rearrangements, exchanges, and endoreduplications, which are seen in less than 10% of the cells from normal persons but in a much higher percentage in cells from FA homozygotes. The karyotypic findings in peripheral blood lymphocytes are presumably representative of all body cells which constitutively manifest identical chromosomal instability.

Whereas chromosome breakage is increased in baseline studies of lymphocytes, it is strikingly enhanced compared with controls if clastogenic agents such as diepoxybutane (DEB) are added to the cultures [9]. Indeed, homozygote Fanconi cells are hypersensitive to many oncogenic and mutagenic inducers, such as ionizing radiation, SV40 viral transformation, and alkylating and chemical agents including mitomycin C, cyclophosphamide, nitrogen mustard, and platinum compounds [1]. For definitive diagnostic purposes, the International Fanconi Anemia Registry (IFAR) based at The Rockefeller University, New York, has defined FA as “increased numbers of chromosome breaks/cell after exposure to DEB [9, 10] with a mean of 8.96 (range 1.3-23.9) compared to normal controls of 0.06 (range 0-0.36). Of note, 10% of IFAR patients had a clonal karyotypic finding rather than uniform breakage with DEB.

The karyotype data, the defects in DNA repair, and the cellular damage that occur in FA translate into an enormous propensity for malignancy. More than 60 patients have been reported with leukemia, 30 with liver tumors, and 30 with other cancers, giving an overall incidence of malignant transformation of about 20% [11]. Fanconi patients may also develop MDS. These are traditionally defined as clonal refractory cytopenias with characteristic dysplastic changes in marrow cells and a propensity to evolve into AML. Some patients also have clonal cytogenetic findings in marrow cells without overt MDS/AML. The clonal findings of deletions, translocations, and marker chromosomes often involve chromosomes 1 and 7 [11].

To determine the risk of malignant myeloid transformation in FA patients, Butturini et al. used the observational database of the IFAR in which most patients' diagnoses were confirmed by DEB testing [12]. This database is a valuable resource for this purpose because of the large number of patients and long follow-up. However, as with any registry, there are potential limitations of the data such as selective reporting and problems with completeness and accuracy. Nevertheless, their analysis provides useful information. For their report, they defined MDS as 5% to 30% myeloblasts in marrow or 5% to 20% myeloblasts in blood. AML was defined as more than 30% marrow blasts or more than 20% blasts in blood. Marrow dysplastic morphology was not used as a criterion.

Of 388 subjects in the IFAR, 332 developed varying hematologic abnormalities at a median of seven years. Of the 332, 59 patients (18%) developed MDS or AML with a median interval of observation prior to the transformation of 13 years (1 month to 32 years). Using the authors' strict disease definitions, 34 patients had MDS and 25 had AML. It is noteworthy that 20 of the 59 patients initially presented at diagnosis with established MDS or AML and the diagnosis of FA was made secondarily. Using the same IFAR data, the actuarial risk of MDS or AML developing over time could
be determined. At 5, 10, 20, and 40 years of age, the probability of malignant transformation escalates from less than 5%, to approximately 8%, 25%, and 52%, respectively. The risk of MDS/AML was higher for patients in whom a prior clonal marrow cytogenetic abnormality had been detected. Loss of chromosome 7 (monosomy 7) or rearrangement or loss of 7q, rearrangements of 1p36 and 1q24-34, and rearrangements of 11q22-25 were the most frequently recurring cytogenetic changes.

Thus, FA is the major model of pre-leukemia in the setting of inherited marrow failure. As therapies and clinical management continue to improve and patients live longer, the frequency of transformation to MDS/AML is likely to increase in parallel.

**OTHER SYNDROMES**

**Shwachman-Diamond Syndrome**

Inherited in an autosomal recessive manner, this disorder basically consists of exocrine pancreatic insufficiency and neutropenia, usually with growth failure. Numerous additional features have been described, including metaphyseal dysostosis, epiphyseal dysplasia, immune dysfunction, liver disease, renal tubular defects, insulin-dependent diabetes mellitus, and psychomotor retardation [13]. Although neutropenia is one of the features, other hematological manifestations have been associated with this disorder. These include anemia, raised fetal hemoglobin, thrombocytopenia, impaired neutrophil chemotaxis [14-16] and aplastic anemia [17], and, like other inherited bone marrow failure syndromes, there is a predilection to leukemic transformation [18-22]. The gene responsible for such a complex and pleiotropic phenotype is not known, and no unifying pathogenesis has been demonstrated that can account for all of the features of the Shwachman-Diamond syndrome.

Extensive experience in Toronto with 25 patients [23] has underscored the clinical importance of the varied phenotype of Shwachman-Diamond syndrome, and the absence of some of the classical findings should not preclude the diagnosis. The vast majority of patients are diagnosed in infancy with symptoms of steatorrhea and poor growth, with and without hematologic abnormalities. The mean birth weight of these patients was at the 25th percentile, but by six months of age mean heights and weights declined to less than the 5th percentile.

Neutropenia occurs on at least one occasion in this disorder but may be intermittent, cyclic, or chronic. Total white-cell counts are often low with the neutropenia; occasionally, total white-cell counts are low in the presence of normal neutrophil counts. Anemia also occurs in more than one-third of patients and thrombocytopenia in 20%. An early but informative study of marrow function was performed in which granulopoiesis was analyzed in 10 children from the Toronto series [24]. Marrow proliferative activity was normal as assessed by determination of mitotic indices and tritiated thymidine uptake into granulocytic cells. Assay of bone marrow colony forming units-granulocyte-macrophage (CFU-GM) progenitors in a methylcellulose tissue culture system demonstrated normal numbers in four patients and reduced numbers in five. The granulocyte colonies were indistinguishable from normal colonies morphologically. Production of “colony-stimulating activity” from patients’ peripheral blood leukocytes appeared normal when tested on control marrow. No serum inhibitors against CFU-GM or “colony-stimulating activity” could be demonstrated using both control and autologous marrow, and coculture of patients’ peripheral blood lymphocytes with control marrow did not inhibit CFU-GM growth. Thus, in Shwachman-Diamond syndrome, committed granulocytic progenitors are proliferative and their frequency in vitro varies widely, as does the clinical neutropenia. The proliferative activity of mitotic granulocytic cells is normal, and neither a deficiency of humoral stimulators nor the presence of serum or cellular inhibitors of granulopoiesis can be demonstrated.

In a recent report of a series of 21 patients from London [25], MDS developed in seven cases (33%). Five of these patients ultimately evolved into AML (M6 in two, M5 in two and M2 in one) following a period of MDS (RAEBT in two, RA in two, and RAEB in one). During the MDS phase, five cases had clonal marrow cytogenetic abnormalities, mostly structural changes involving chromosome 7. In the Toronto series [23], 11 of the 25 patients had pancytopenia and three of these developed AML (an incidence of 12%). In published literature of 165 patients [11], nine developed leukemia (5%): three cases of acute lymphoblastic leukemia, two of AML, one of AMML, one of AMoL, one of EL, and one of JCML. These sporadic reports are likely a gross underestimate of the true incidence of malignant transformation, judging from the London and Toronto data. Clearly, the propensity for leukemic conversion in Shwachman-Diamond syndrome is extremely high compared with the general population but is probably not as high as in Fanconi’s anemia.

**Diamond-Blackfan Syndrome**

Although not generally regarded as a pre-leukemic condition, Diamond-Blackfan anemia (congenital red-cell aplasia, or DBA) is also associated with malignant myeloid transformation. DBA is a disorder that is heterogeneous with respect to inheritance patterns, clinical and laboratory findings, in vitro data, and therapeutic outcome [26-28]. About 80% of cases are sporadic, suggesting new mutations or acquired disease, but there are examples of recessive inheritance (autosomal and possibly X-linked), as well as autosomal dominant patterns. There is a growing suspicion that
DBA represents a family of disorders with different molecular etiologies that share the common hematological phenotype of red-cell aplasia.

The uniform diagnostic criteria for all cases are: (A) normochromic-macrocytic anemia presenting in 90% of cases in the first 12 months of life; (B) profound reticulocytopenia; (C) normocellular marrow with a selective, marked deficiency of red-cell precursors; (D) increased serum levels of erythropoietin; (E) normal or slightly decreased white cell counts, and (F) normal or increased platelet counts. Fetal hemoglobin is usually increased with decreased white cell counts, and (F) normal or increased serum levels of erythropoietin; (E) normal or slightly marked deficiency of red-cell precursors; (D) increased cases in the first 12 months of life; (B) profound reticulo-normochromic-macrocytic anemia presenting in 90% of red-cell aplasia.

Lar et al. identified any of these putative abnormalities. Although these new data broaden our understanding of the DBA cellular defect, the many previous studies using clonogenic assays underscored that DBA erythroid progenitors are unable to respond normally to inducers of erythroid proliferation and/or differentiation. Confirmation of this was demonstrated by showing that CD34+ DBA progenitors differentiated normally in short-term clonogenic assays along megakaryocytic and granulocytic pathways but aberrantly along the erythroid lineage [30]. Accelerated programmed cell death (apoptosis) may play a role in this pathogenesis but requires further study. Based on the various patterns of erythroid colony growth seen with DBA patients, a model for the aberrant erythropoiesis was developed that proposes maturational arrests at varying sites along the differentiation pathway [27]. Recent studies indicate that recombinant interleukin 3 (rIL-3) and Steel factor in combination with EPO may increase the in vitro clonogenicity of DBA bone marrow progenitors from post-Percoll cell preparations [26]. These findings have raised speculation that DBA is due to one or more receptor-ligand abnormalities involving various growth-promoting cytokines. Thus far, studies have failed to identify any of these putative abnormalities.

As summarized by Lipton and Alter [28], acute leukemia and/or MDS have been reported in eight patients with DBA. One girl developed acute lymphoblastic leukemia at age 13 after a spontaneous remission of DBA at age five; the leukemia also remitted completely with therapy, and neither disorder was present at age 17. Two patients originally described by Louis Diamond had intermittent remissions of DBA but died of AML at ages 31 and 43, respectively. One of them had received thymic and skeletal irradiation during childhood as “therapy” for DBA. A girl who received cyclophosphamide for treatment of DBA died of acute promyelocytic leukemia at age 13. A boy who developed acute megakaryoblastic leukemia at age 14 months had anemia at two months of age; this may have been a long preleukemic phase. Three male steroid-nonresponders developed MDS at ages 13, 21, and 22 years, respectively. One evolved into AMML, one into AML and the third patient died of complications of MDS.

Thus, of 379 published cases of DBA [11], the number of cases of malignant transformation (eight cases, 2% incidence) is inordinately high and may even be higher as new cases [31] are diagnosed and reported. The link between disordered erythropoiesis and myeloid malignant disease is somewhat clearer now with the advent of new information described herein, implicating an earlier marrow progenitor in the pathogenesis of DBA than was previously appreciated [29].
Congenital Amegakaryocytic Thrombocytopenia (CAT)

CAT is a varied syndrome that presents with isolated thrombocytopenia due to reduced or absent marrow megakaryocytes in early life, often within the first week. Although the hematologic phenotype is similar, some patients have a normal physical appearance whereas others have anomalies of diverse nature. As reviewed by Alter [11], the distinction between those with and those without anomalies is arbitrary; the inheritance patterns in both groups, when they are interpretable, suggest X-linked or autosomal recessive transmission of disease, but sporadic cases occur as well.

Serial studies of bone marrow hemopoiesis using clonogenic assays were performed in an infant from Toronto with CAT [32]. Initially, when the only hematological abnormality was isolated thrombocytopenia, the number of clonogenic hematopoietic progenitors was comparable to controls, including the number of megakaryocyte precursors. As the disease evolved into aplastic anemia over an 11-month period, the peripheral blood counts declined, and colony numbers from four classes of progenitors (BFU-E, CFU-GM, colony forming unit-mixture (CFU-MIX), and colony forming unit-megakaryocytic (CFU-MEG) also declined in parallel. When added to the marrow cultures, the patient’s plasma was not inhibitory to either control or to colony growth. Similarly, no cellular inhibition of hemopoiesis was observed when the patient’s marrow was cultured after depleting the sample of T lymphocytes or after adding them back. Furthermore, stromal cells established from short-term and long-term cultures of the patient’s marrow showed normal proliferative activity and yielded a “fertile” marrow microenvironment for both the patient’s and the control’s colony growth. The data suggest that the central problem in CAT is an intrinsic hematopoietic stem cell defect rather than an abnormality of the marrow milieu. The findings are consistent with either a progressive, quantitative attrition of progenitors or their inability to proliferate into colonies in vitro and into differentiated, functional cells in vivo.

Patients can have persistent, usually severe thrombocytopenia, but about 45% evolve into full-blown aplastic anemia. Alter described two patients with CAT that developed leukemia [11]. One male with a normal physical appearance had amegakaryocytic thrombocytopenia from day 1, developed aplastic anemia at age five, responded poorly to androgens and steroids, and then evolved into AMML at age 16, with death at age 17. A female had thrombocytopenia at age two months, pancytopenia at five months, and a preleukemic picture with abnormalities involving chromosome 19. The Toronto patient described herein had thrombocytopenia at six months, progressive aplastic anemia over the next two years, monosomy 7 in marrow cells by five years and then MDS with an activating ras oncogene mutation in hematopoietic cells [33]. Therefore, the current evidence shows that CAT is another inherited marrow failure disorder that is pre-leukemic. The risk or incidence of malignant conversion is difficult to determine because of the rarity of the disease and paucity of published data dealing with this issue.

Congenital Neutropenia and Kostmann’s Syndrome (KS)

Severe chronic neutropenia and recurrent serious infections are features of a heterogeneous group of disorders of myelopoiesis including congenital neutropenia, cyclic neutropenia, and idiopathic neutropenia. KS is a subtype of congenital neutropenia inherited in an autosomal recessive manner with onset in early childhood, profound neutropenia (absolute neutrophil count <200/ml), recurrent life-threatening infections, and a maturation arrest of myeloid precursors at the promyelocyte-megakaryocyte stage of differentiation. Until recently, most patients with KS died early in life from infections. Congenital neutropenia and KS have the same hematological phenotype and clinical presentation. The recessive inheritance of KS is made by inference when there is more than one affected child in a family. Congenital neutropenia is the proper designation used for a single “sporadic” case in a family, and hence may or may not be inherited in an autosomal recessive manner like KS. Since the molecular defect is not known for either diagnostic category, the ability to “lump” or “split” the two disorders remains a subject of argument.

Administration of pharmacologic doses of rhG-CSF to patients with various forms of chronic neutropenia, including the congenital types, induces a marked increase in circulating neutrophil counts and is associated with a significant reduction in serious infections and improved quality of life. Although the fundamental basis of most cases of chronic neutropenia and the reasons that affected individuals show a therapeutic response to G-CSF are largely unknown, the available data are consistent with an intrinsic defect of immature myeloid cells rather than impaired endogenous G-CSF production.

Prior to the availability of G-CSF for therapeutic purposes, it was recognized that leukemic transformation occurred occasionally in patients with congenital neutropenia [11, 34-37]. However, in the pre-cytokine era, many congenital neutropenia patients died in the first years of life from other causes. Of the published cases, 42% of patients died at a mean age of two years secondary to sepsis and pneumonia [11]. Thus, the true risk of congenital neutropenia patients developing MDS/AML was not defined. Currently with G-CSF therapy, most of these patients do not develop life-threatening infections and are surviving, but it is not known if longer survival will allow for the natural expression of leukemogenesis in this population. Moreover, since the long-term effects of G-CSF are not
known, it is still unclear whether MDS or AML will occur with increased frequency in patients who receive prolonged therapy of G-CSF to correct the neutropenia.

This serious question has prompted the development of the Severe Chronic Neutropenia International Registry based at the University of Seattle, Washington, to monitor patients worldwide with neutropenic disorders who are receiving G-CSF treatment. Current data from the Registry [38] show that 23 of 249 patients with congenital neutropenia (9%), but not other categories of neutropenia, developed MDS/AML while receiving long-term G-CSF therapy. Of the 21, 15 were male; the mean age was 14 (range 4-48), the mean G-CSF dose was 15.3 mg/kg/day (1-62.1), and the mean duration of G-CSF therapy to the onset of MDS/AML was 51.2 months (0-102.1). No relationship between dose or duration of G-CSF was apparent in a careful analysis of the data. Of the 23, marrow from 10 was studied [33] for activating ras oncogene mutations; abnormalities were identified in five after transformation but not before.

To determine differences between patients that do and do not undergo transformation, a study was initiated to identify abnormalities in the function of the G-CSF receptor (G-CSF-R) [39]. Among 25 patients analyzed, five had point mutations in the G-CSF-R gene. The mutations, present in cells of the myeloid lineage only, were nonsense mutations leading to truncation of the C-terminal cytoplasmic region crucial for maturation signaling. In these five cases, both the mutated and the normal alleles were expressed. Three patients developed cytogenetic abnormalities, t(1;5), t(6;9) and monosomy 7, respectively, and developed AML. Of the two other neutropenic patients, one developed monosomy 7. Twenty patients without G-CSF-R mutations, including five Swedish cases with a family history of neutropenia from the original pedigree of Kostmann, showed no cytogenetic or clinical signs of progression to MDS or AML. Thus, a mutated G-CSF-R may identify a subset of patients who will undergo conversion to MDS/AML. Four additional patients with congenital neutropenia have been found with a mutated G-CSF-R gene but have not developed MDS/AML. The clinical outcome of this group is obviously under close surveillance.

Leukemogenesis in this population is a complex, multistep process characterized by a series of cellular genetic changes which are, as yet, poorly understood. The stepwise acquisition of monosomy 7, ras oncogene mutations, and G-CSF receptor mutations in some patients with congenital neutropenia clearly indicate a genetic predisposition to malignant transformation. The role, if any, of G-CSF in leukemogenesis or in the development of cytogenetic abnormalities in this group of patients remains unknown.

**CONCLUSIONS AND SPECULATION**

Carcinogenesis occurs as a sequence of events that is driven by genetic damage and by epigenetic changes. In the traditional view, the initiation of cancer starts in a normal cell through mutations from exposure to carcinogens. In the promotion phase that follows, the genetically altered, initiated cell undergoes selective clonal expansion that enhances the probability of additional genetic damage from endogenous mutations or DNA-damaging agents. Finally, during cancer progression, malignant cells show phenotypic changes, gene amplification, chromosomal alterations and altered gene expression.

In the inherited marrow failure syndromes described herein, the first “hit” or cancer-initiating step may be the constitutional genetic abnormality itself that initially manifests as the single-lineage or multiple-lineage myelopathy. The “predisposed” progenitor, already initiated, could conceptually show decreased responsiveness to the signals that regulate homeostatic growth, for example, less responsive to negative growth factors, terminal cell differentiation, or programmed cell death. The leukemic promotion and progression steps leading to MDS/AML could then ensue readily in the initiated pool of progenitors or stem cells. When the molecular defects that produce the marrow failure syndromes are discovered, the nature of the leukemogenic-initiating events in these conditions should become evident.

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**REFERENCES**


