

CONGENITAL MARROW FAILURE AND MALIGNANT HEMATOPOIETIC TRANSFORMATION

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Introduction

Bone marrow failure is defined as decreased production of one or more of the major hematopoietic lineages due to congenital or acquired factors, or both. With regard to the “congenital” disorders, the inference is that the aberrant hematology is present at birth, but this is not always the case. “Inherited” is a better designation since the genetic patterns of inheritance are well defined for many of these conditions. Since 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 disorders, the notion is advanced that genetic mutations that interfere with hematopoiesis account for the marrow failure, although the specific molecular basis is not yet known for any of these conditions.

Historically, the inherited marrow failure syndromes were classified as “benign” hematology in contrast to the “malignant” disorders such as leukemia. Patients with severe clinical forms of these syndromes such as Kostmann’s syndrome or congenital neutropenia, Shwachman-Diamond syndrome, Fanconi’s anemia, and 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, administration of recombinant cytokines, growth factors and other therapeutics, patients with these conditions usually survive the early years of life and beyond. With the extended life span of patients, we are now observing a new natural history for some of these disorders. 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 both (MDS/AML). Thus, the distinction between “benign” and “malignant” hematology in the context of inherited marrow failure disorders has become blurred, and a new clinical and hematological continuum must be described.

Fanconi’s Anemia

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

The basic defect in Fanconi’s anemia is unknown. Although at least five genes have been demonstrated by complementation studies, only two have been definitively

identified so far (type A and type C) and the role of their protein products is still unclear. The two theories of pathogenesis of Fanconi's anemia relate to either defective DNA repair or an inability of Fanconi cells to remove oxygen free radicals that damage cells. The confirmatory laboratory test is the abnormal chromosome pattern seen in metaphase preparations of phytohemagglutinin-stimulated cultured peripheral blood lymphocytes. These reveal breaks, gaps, rearrangements, exchanges, and endoreduplications, which are seen in less than 1% of the cells from normal persons but much higher in cells from Fanconi's anemia 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 to controls if clastogenic agents such as diepoxybutane (DEB) are added to the cultures. 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. For definitive diagnostic purposes, the International Fanconi Anemia Registry (IFAR) based at The Rockefeller University, New York City, has defined Fanconi's anemia as increased numbers of chromosome breaks/cell after exposure to DEB 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 karyotypic data, the defects in DNA repair, and the cellular damage that occur in Fanconi's anemia reflect an enormous propensity for malignancy. More than 60 patients have been reported with leukemia, 30 with liver tumours, and 30 with other cancers, giving an overall incidence of malignant transformation of about 20%.⁽¹⁾ Fanconi patients may also develop MDS. These are defined as clonal refractory cytopenias with characteristic dysplastic changes in marrow cells in at least two of the three hematopoietic lineages 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 chromosome 1 and 7.

Review of the IFAR data in 1992 revealed 313 Fanconi patients with varying hematologic changes; 53 (17%) of these developed MDS/AML with a median interval of observation prior to this transformation of 12 years.⁽²⁾ Of note, 18 of the 53 initially presented at diagnosis with established MDS or acute leukemia. Using the IFAR data, the actuarial risk of developing MDS/AML in Fanconi's anemia over time can be plotted. At 5, 10, 20 and 30 years of age, the probability of malignant transformation escalates from less than 5%, to 8%, 25% and 50%, respectively. As therapies and clinical management continue to improve and Fanconi 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. Extensive

experience in Toronto with 25 patients⁽³⁾ has underscored the importance of an extremely varied phenotype at diagnosis. The absence of some of the classical findings does not preclude the diagnosis. The vast majority of patients are diagnosed in infancy with symptoms of steatorrhea and poor growth, with or without hematologic abnormalities. Less common manifestations also may be evident, such as skeletal abnormalities and marked hepatomegaly. Exocrine pancreatic dysfunction, in variable degrees of severity, is a universal feature; however, the diagnosis must still be considered even if clinical symptoms of pancreatic insufficiency are absent since a significant percentage of patients develop pancreatic sufficiency with time or are pancreatic sufficient at diagnosis.

Neutropenia occurs on at least one occasion in these patients but it 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 studied in 10 children from the Toronto series with Shwachman-Diamond syndrome.⁽⁴⁾ Marrow proliferative activity was normal as assessed by determination of mitotic indices and tritiated thymidine uptake into granulocytic cells. Assay of bone marrow 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 co-culture of patients' peripheral blood lymphocytes with control marrow did not inhibit CFU-GM growth. Thus, in Shwachman-Diamond syndrome, committed granulocytic progenitors are present, and the numbers assayed in vitro vary widely as does the clinical neutropenia. The proliferative activity of recognizable 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 the current Toronto series,⁽³⁾ 11 of the 25 patients had pancytopenia and three of these developed AML (an incidence of 12%). In published literature of 165 patients,⁽¹⁾ nine developed leukemia (5%): three cases of acute lymphoblastic leukemia, two of AML, and one each of AMML, AMoL, EL, and JCML. Clearly, the propensity for leukemic transformation in Shwachman-Diamond syndrome is extremely high compared to the general population but not as high as in Fanconi's anemia. The genetic mutation that accounts for this marrow instability and dysfunction as well as the other pleiotropic manifestations of this disorder remains elusive.

Diamond-Blackfan Syndrome

Although not generally known, Diamond-Blackfan anemia (congenital red cell aplasia or DBA) is also associated with malignant hematopoietic transformation. DBA is a disorder that is heterogeneous with respect to inheritance patterns, clinical and laboratory findings, in vitro data, and therapeutic outcome. 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: ⁽¹⁾ normochromic-macrocytic anemia presenting in 90% of cases in the first 12 months of life, ⁽²⁾ profound reticulocytopenia, ⁽³⁾ normocellular marrow with a selective, marked deficiency of red cell precursors, ⁽⁴⁾ increased serum levels of erythropoietin, ⁽⁵⁾ normal or slightly decreased white cell counts, and ⁽⁶⁾ normal or increased platelet counts. Fetal hemoglobin is usually increased with a fetal Gg/Ag pattern, is distributed heterogeneously, and is associated with increased expression of red cell i antigen as well as with fetal levels of red cell glycolytic and hexose monophosphate shunt enzyme activities. Erythrocyte adenosine deaminase (ADA), an enzyme in the purine salvage pathway, is increased in 90% of DBA patients. Since increased ADA activity has also been demonstrated in several other benign and malignant marrow disorders affecting progenitor cells, there may be a relationship between elevated ADA levels and intrinsically abnormal DBA erythroid progenitors.

The cellular basis for most cases of DBA is becoming clearer. ⁽⁵⁾ Initial reports of humoral, cellular or microenvironmental inhibitors of erythropoiesis in DBA could not be confirmed. A large body of evidence indicates that the erythroid progenitor compartment is intrinsically defective in DBA. Cultures of DBA marrow using standard clonogenic assays for CFU-E and BFU-E progenitors consistently have shown reduced or absent colonies in most DBA patients, and intermediate, normal or occasionally increased numbers in the rest. The DBA erythroid progenitors are relatively insensitive to erythropoietin (EPO) in vitro and to 'burst-promoting activity', but the hyporesponsiveness to EPO can be corrected in some cases by the addition of glucocorticoids in vitro or by clinically administering prednisone.

The data underscore that the intrinsic defect of DBA erythroid progenitors is an inability to respond normally to inducers of erythroid proliferation and/or differentiation. Confirmation of the overall defect was demonstrated by showing that CD34+ DBA progenitors differentiated normally along megakaryocytic and granulocytic pathways but aberrantly along the erythroid lineage. 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. ⁽⁶⁾ Recent studies indicate that recombinant interleukin-3 (IL-3) and Steel factor (SCF) in combination with EPO may increase the in vitro clonogenicity of DBA bone marrow progenitors from unfractionated cell preparations. 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, ⁽⁷⁾ 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 5; the leukemia also remitted completely, and neither disorder was present at age 17. Two patients originally described by Diamond et al. 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 2 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 acute myelomonocytic and one into acute myeloid leukemia, and the third patient died of complications of MDS.

Thus, of 379 published cases of DBA,⁽¹⁾ eight cases of malignant transformation (2% incidence) is inordinately high. The link between disordered erythropoiesis and myeloid malignant disorders is a puzzling but important research challenge.

Congenital Amegakaryocytic Thrombocytopenia

Congenital amegakaryocytic thrombocytopenia (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,⁽¹⁾ 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 hematopoiesis using clonogenic assays were performed in an infant from Toronto with CAT.⁽⁸⁾ 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, CFU-Mix, and CFU-Meg) also declined in parallel. When added to the marrow cultures, patient’s plasma was not inhibitory to either control or to patient’s colony growth. Similarly, no cellular inhibition of hematopoiesis was observed when patient’s marrow was cultured after depleting the sample of T lymphocytes and after adding the T lymphocytes back. Furthermore, stromal cells established from short-term and long-term cultures of patient’s marrow showed normal proliferative activity and yielded a “fertile” marrow microenvironment for patient’s and control 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 who developed leukemia.⁽¹⁾ One male with a normal physical appearance had amegakaryocytic thrombocytopenia from day 1, developed aplastic anemia at age 5 years, responded poorly to androgens plus steroids, and then evolved into acute myelomonocytic leukemia at age 16, with death at age 17. A female had thrombocytopenia at age 2 months, pancytopenia at 5 months, and a preleukemic picture with abnormalities involving chromosome 19. The Toronto patient described above had thrombocytopenia at 6 months, progressive

aplastic anemia over the next 2 years, monosomy 7 by 5 years and then MDS with an activating ras oncogene mutation in hematopoietic cells. Therefore, the current evidence is strong that CAT is another inherited marrow failure disorder that is pre-leukemic.

Severe Chronic Neutropenia and G-CSF Therapy

Severe chronic neutropenia and recurrent serious infections are features of a heterogeneous group of disorders of myelopoiesis including congenital agranulocytosis (Kostmann's syndrome [KS]), cyclic neutropenia, and idiopathic neutropenia. The bone marrows of affected patients typically show a paucity of mature myeloid cells. 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-myelocyte stage of differentiation. Until recently, most patients with KS died early in life from infections.

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

Patients with a variety of inherited and acquired conditions, including occupational or medical exposure to mutagens, aplastic anemia, paroxysmal nocturnal hemoglobinuria, and the inherited disorders described herein are predisposed to MDS/AML. Molecular analysis has shown acquired mutations at codons 12, 13, or 61 of the K ras or N ras proto-oncogenes in 20% to 30% of adults and children with preleukemia and AML. In addition, the bone marrows of many patients with MDS and AML acquire nonrandom cytogenetic abnormalities, including specific chromosomal translocations, duplications, and deletions. Loss of all or part of chromosomes 5 and 7 are particularly common in cases of MDS and AML that develop in patients with known genetic or occupational predispositions. Although AML had been reported in three patients with KS who never received rhG-CSF, many affected children die during the first few years of life, and the association between KS and myeloid leukemia has been incompletely defined. Moreover, the long-term effects of rhG-CSF are not known. In particular, it is unclear whether MDS or AML will occur with increased frequency in patients who receive prolonged therapy of rhG-CSF to correct the neutropenia.

This 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 rhG-CSF treatment. Current data from the Registry⁽⁹⁾ show that about 10% of patients with congenital neutropenia or KS but not other categories of neutropenia develop MDS/AML while receiving long-term rhG-CSF therapy. Some of these cases are included in the detailed report of 13 patients with congenital disorders of myelopoiesis who developed MDS or AML while receiving rhG-CSF therapy.⁽¹⁰⁾ Bone marrows of 10 of these patients showed monosomy 7, and

five had activating ras oncogene mutations. One additional patient acquired a clonal cytogenetic abnormality in marrow cells while receiving rhG-CSF without evidence of MDS/AML. These abnormalities were not detected in pretreatment bone marrows, and cessation of rhG-CSF was not associated with either clinical improvement or cytogenetic remission. Thus, patients with severe forms of congenital neutropenia are at relatively high risk of developing MDS and AML. The occurrence of monosomy 7 and ras mutations in these cases suggests that the myeloid progenitors of some patients are genetically predisposed to malignant transformation. The relationship between therapeutic rhG-CSF and leukemogenesis in patients with severe chronic neutropenia is unclear and requires close surveillance.

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) in severe chronic neutropenia.⁽¹¹⁾ 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 acquired cytogenetic abnormalities, i.e., t(1;5), t(6;9) and monosomy 7, respectively, and developed AML. Of the two other patients, still neutropenic, one acquired 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.

References

1. Alter BP. Inherited bone marrow failure syndromes. In: Handin RI, Stossel TP, Lux SE, eds. *Blood: Principles and Practice of Hematology*. Philadelphia: Lippincott 227-91. 1995.
2. Schwartz E, Young N, Freedman MH, Gillio A. Failure of erythropoiesis: Causes and treatments. In: *Hematology 1992, Education Program of American Society of Hematology* 1-7. 1992.
3. Mack DR, Forstner GG, Wilschanski M, Freedman MH, Durie PR. Shwachman syndrome: Exocrine pancreatic dysfunction and variable phenotypic expression. Submitted.
4. Saunders EF, Gall G, Freedman MH. Granulopoiesis in Shwachman's syndrome (pancreatic insufficiency and bone marrow dysfunction). *Pediatrics* 64: 515. 1979.
5. Freedman MH. Annotation. Pure red cell aplasia in childhood and adolescence: Pathogenesis and approaches to diagnosis. *Br J Haematol* 85: 246. 1993.
6. Halperin DS, Freedman MH. Diamond-Blackfan anemia: Etiology, pathophysiology and treatment. *American J Ped Hematol-Oncology* 11: 380. 1989.
7. Lipton JM, Alter BP. Diamond-Blackfan anemia. In: Feig SA, Freedman MH (eds.) *Clinical Disorders and Experimental Models of Erythropoietic Failure*. Boca Raton: CRC Press 39-68. 1993.

8. Freedman MH, Estrov Z. Congenital amegakaryocytic thrombocytopenia: An intrinsic hematopoietic stem cell defect. *Am J Ped Hematol-Oncol* 12: 225. 1990.
9. Dale DC, Cottle T, Bolyard AA, Fier C, Bonilla MA, Boxer L, Brown SL, Cham B, Freedman MH, Kannourakis G, Welte K. Severe chronic neutropenia: Report on treatment and outcome from a new international registry. *Blood (Supplement 1)*: 425a. 1995.
10. Kalra R, Dale D, Freedman MH, Bonilla MA, Weinblatt M, Ganser A, Bowman P, Abish S, Priest J, Oseas RS, Olson K, Paderanga D, Shannon K. Monosomy 7 and activating ras mutations accompany malignant transformation in patients with congenital neutropenia. *Blood* 86: 4579. 1995.
11. Touw IP, de Koning JP, Lowenberg B, Dong F. Defective G-CSF receptors in severe congenital neutropenia (SCN) associated with progression towards MDS and AML. *Blood* 86 (Supplement 1): 26a. 1995