

Bone marrow failure in children

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Bone marrow failure in the pediatric patient places the hematologist at the junction of clinical medicine, cellular biology, and molecular genetics. The pathophysiology of these disorders is rapidly being elucidated in many laboratories. Treatments such as bone marrow transplantation and the nascent modality of gene therapy are firmly grounded in these modern sciences. This year's progress in the understanding, diagnosis, and treatment of a wide variety of predominantly pediatric bone marrow failure states is a direct result of the union between the "laboratory bench and the patient bedside."

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Current Opinion in Pediatrics 1996, 8:33-41

Abbreviations

ATG	antithymocyte globulin
BMT	bone marrow transplantation
DBA	Diamond Blackfan anemia
FA	Fanconi anemia
FACC	Fanconi anemia complementation group C
G-CSF	granulocyte colony-stimulating factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
GVHD	graft-versus-host disease
SAA	severe aplastic anemia
TEC	transient erythroblastopenia of childhood

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ISSN 1040-8703

Disorders of bone marrow failure defined as aplastic anemia and the single cytopenias, in particular the congenital syndromes, are a *sine qua non* of pediatric hematology. Although extraordinarily rare, an understanding of the pathophysiology of bone marrow failure has provided significant insights relevant to the more common situations of hematopoietic malignancy, chemotherapy-induced cytopenia, and hematopoietic stem cell engraftment. As well, these disorders provide the "experiments of nature" that elucidate the progenitor model of hematopoiesis illustrated in Figure 1. In this model, the pluripotent stem cell gives rise to the full array of committed progenitors that populate the bone marrow with recognizable precursors of the functional hematopoietic cells ultimately released into the peripheral circulation and tissues. That this process of hematopoietic cell self-renewal, commitment, differentiation, and amplification exists in a highly regulated microenvironment consisting of stroma, accessory cells, and growth factors is now well established. Thus the pathophysiology of bone marrow failure can be subjected to multiple hypotheses, attributing the flaw in blood cell production to one of the potential defects listed in Table 1. Moreover, current evidence supports bone marrow failure as a heterogeneous group of disorders with diverse pathophysiology.

Recent advances in cellular and molecular biology have added considerably to the understanding of hematopoietic cell growth and the highly complex regulatory network supporting this process. In addition, clinical studies have used this new knowledge to develop improved therapies for patients with the bone marrow failure conditions outlined in Tables 2 and 3. In this review we cite what we believe are the most important articles, published between August 1994 and July 1995, describing clinical and laboratory advances in the treatment and biology of bone marrow failure. Where necessary, earlier articles are cited in order to provide essential background. Unfortunately, space limitations permit neither an exhaustive review of all the marrow failure syndromes nor a summary of the exciting advances in basic science that will lay the groundwork for future studies. Those interested in more extensive reading are directed to Young and Alter's [1] recent comprehensive text as well as review articles cited throughout this paper.

Aplastic anemia

Severe aplastic anemia

Aplastic anemia is defined as trilineage hematopoietic failure resulting in anemia, neutropenia, and thrombocytopenia. In severe cases the pancytopenia is profound and

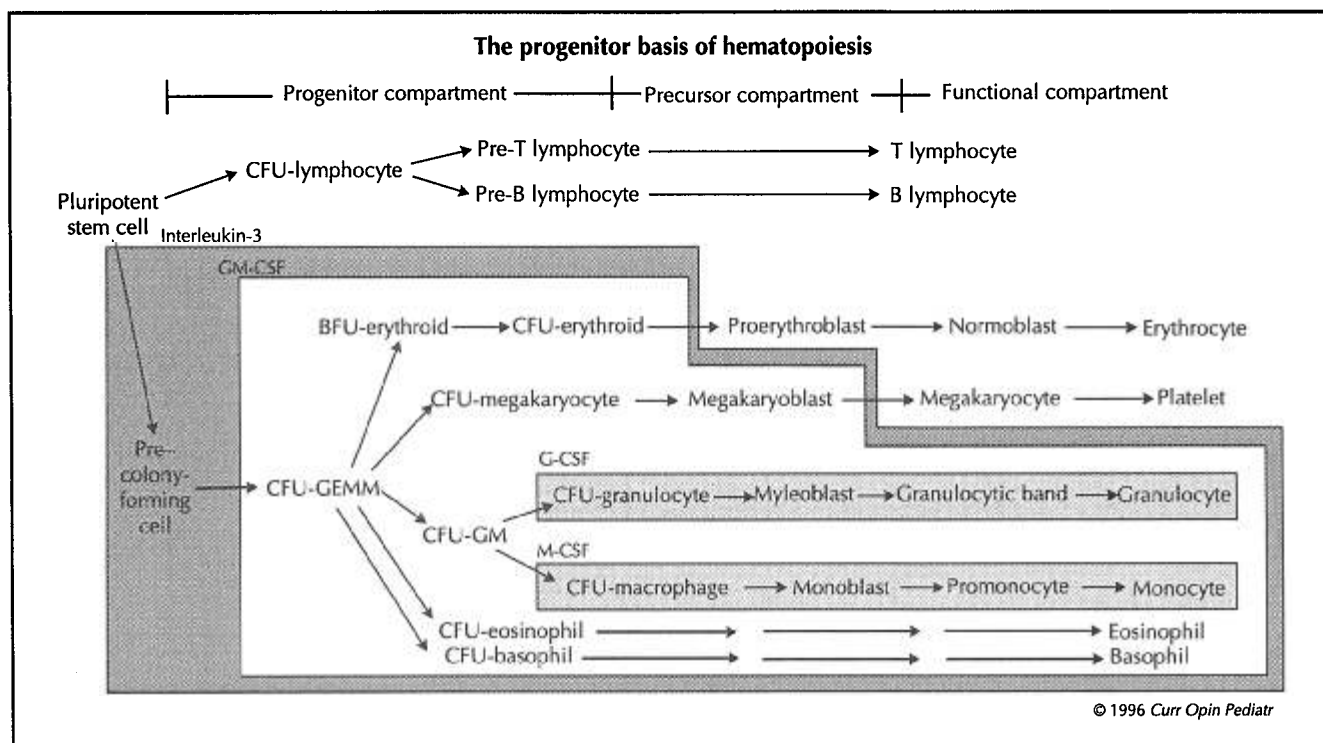


Fig. 1. The progenitor basis of hematopoiesis. Schematic diagram as defined by clonal assays and flow cytometric analysis in the human and murine system and studies in diseases of clonal proliferation. Enclosed areas define the effect of the colony-stimulating factors interleukin-3, GM-CSF (granulocyte-macrophage colony-stimulating factor), G-CSF (granulocyte colony-stimulating factor), and M-CSF (macrophage colony-stimulating factor). The hierarchy of hematopoietic cell differentiation proceeds from the pluripotent stem cell through the progenitor compartment, cells that are not morphologically identifiable but have undergone commitment, to the morphologically identifiable cells of the precursor compartment, which give rise to the mature cells of the functional compartment. BFU—burst-forming unit; CFU—colony-forming unit; GEMM—granulocyte, erythrocyte, megakaryocyte, macrophage.

the bone marrow is markedly hypocellular. The result, in cases unmodified by therapy, is transfusion dependence and, frequently, death from infection or bleeding. Allogeneic bone marrow transplantation (BMT) from an HLA-identical sibling has been considered the treatment of choice for severe aplastic anemia (SAA) since the late 1970s. An analysis of over 2000 patients in Europe was performed by Bacigalupo [2•] and the European Group of BMT. Of 18 syngeneic (identical twin) transplants performed in Europe, conditioned with cyclophosphamide, 17 are alive with a median follow-up of 5 years. Improvement has also been noted for HLA-matched sibling transplants with an increase in actuarial survival, from 37% in the decade 1970 to 1979 to 68% in the decade 1984 to 1993. This improvement has been attributed to early intervention and a significant reduction in graft rejection and graft-versus-host disease (GVHD) using cyclophosphamide for pretransplantation conditioning and adding cyclosporine for GVHD prophylaxis, respectively.

Storb [3] reviewed data on the preparative regimen for patients with SAA undergoing BMT at the Fred Hutchinson Cancer Research Center in Seattle. Because the bone marrow space is presumed to be "empty" in these patients, conditioning involves immunosuppression rather than myeloablation. Initially, cyclophosphamide

was used at 50 mg/kg/d for 4 consecutive days. Graft rejection, although rare in previously untransfused patients, was seen in 35% of the Seattle patients, with an event-free survival of only 45%. Untransfused patients being in the minority, the investigators in Seattle then undertook a study in which buffy coat cells harvested from donor peripheral blood were infused to augment the graft. This approach markedly decreased rejection and increased the survival rate of the transfused patients to 70%. However, the incidence of chronic GVHD greatly increased. Other centers demonstrated that regimens using either limited field or total body irradiation combined with cyclophosphamide enhanced engraftment without the concomitant increase in GVHD seen in the Seattle study. Because these patients may be at risk for late secondary malignancies, the Seattle group instead used cyclophosphamide with antithymocyte (antilymphocyte) globulin (ATG) for their next group of patients. The authors reported successful sustained engraftment in 30 of 33 patients (91%), many of whom were sensitized by transfusion, at between 1 and 5 years after BMT. The incidence of acute GVHD was 15% and that of chronic GVHD 35%, significantly less than that reported with the use of buffy coat cell transfusions. These results have been replicated by Horstmann *et al.* [4], who performed transplants in nine patients with the same regimen using HLA-identical related donors. Eight had sustained

Table 1**Pathophysiology of marrow failure**

Cellular defects
Absence of or abnormality in the pluripotent or multipotent stem cell
Absence of or abnormality in the committed stem cell
Absence of or abnormality in the precursor cell
Humoral or cellular (immunologic) inhibitors of pluripotent, multipotent, committed, or precursor cells
Defects in or absence of helper cells
Humoral defects
Absence of or defect in stimulator, helper, or humoral regulators
Humoral or cellular (immunologic) inhibitors of these regulators
Stromal (microenvironmental) defects

Modified from Hedberg and Lipton [46].

engraftment with transfusion independence at a median follow-up of 30 months.

The cyclophosphamide-ATG regimen, when used in patients undergoing unrelated donor transplants, resulted in failures due to early rejection or unsustained engraftment [5]. Given these data and the observation that less than a third of patients have a suitable HLA-matched donor, the use of immunomodulation to treat SAA has expanded dramatically since it was introduced in the 1970s. Rosenfeld *et al.* [6] studied the effect of ATG with cyclosporine on 51 patients of all ages with SAA; 67% responded by 3 months and 78% by 1 year. The relapse rate was high at 36% at 2 years, but most patients responded to retreatment. One patient progressed to acute leukemia and five patients developed paroxysmal nocturnal hemoglobinuria. Bacigalupo [2•] reported an overall 5-year actuarial survival of 43% for patients treated with immunomodulation between 1970 and 1984 and 66% for those patients treated thereafter. In Europe, immunomodulation using ATG in combination with methylprednisolone and cyclosporine has been reported to be superior to ATG alone. Recently either recombinant human granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF) has been added to most SAA treatment regimens in an effort to improve neutrophil counts until there is definitive marrow recovery. In a recent European Group of BMT study, 40 patients with newly diagnosed SAA were treated with horse ATG, methylprednisolone, cyclosporine, and G-CSF [7••]. Three patients (8%) died of sepsis within the first 100 days, all having had initial absolute neutrophil counts less than $0.2 \times 10^9/L$. Four patients (10%) remained transfusion-dependent and were considered nonresponders. Thirty-three patients (82%) become transfusion independent. When grouped by pre-treatment neutrophil counts of less than $0.2 \times 10^9/L$ versus counts of $0.2 \times 10^9/L$ or higher, the proportion of responders was 71% versus 95%, respectively. Forty percent of the patients were partial responders, requiring transfusion support for a median of 215 days, and 42%

showed a complete response and stopped transfusions at a median of 65 days. One of the complete responders needed a second course of ATG, whereas six in the partial responder group needed retreatment. No patient has yet developed a clonal abnormality. Survival for the patients with an initial absolute neutrophil count of less than $0.2 \times 10^9/L$ was 86% as compared with 100% for those with an absolute neutrophil count between 0.2 and $0.5 \times 10^9/L$. Overall survival was 92% with a median follow-up of 428 days. Neither gender nor age influenced survival in this study. The authors postulate that the decrease in early mortality may be due to the increase in neutrophil counts in G-CSF responders.

In a smaller study, Hord *et al.* [8•] used a similar combination of ATG, prednisone, cyclosporine, and GM-CSF in seven children with newly diagnosed SAA. All had an increase in the absolute neutrophil count to more than $1.0 \times 10^9/L$ within 3.5 months of diagnosis. One patient went on to BMT and was removed from the study. Of the six remaining, five are transfusion independent and one remains on therapy with evidence of a decreased transfusion requirement.

The European Group of BMT [2•] compared BMT with immunomodulation and formulated two conclusions. First, in patients with SAA and low absolute neutrophil counts ($\leq 0.5 \times 10^9/L$), BMT and immunomodulation

Table 2**Classification of the aplastic anemias**

Acquired
Secondary
Radiation
Drugs and chemicals
Regular: cytotoxic agents, benzene
Idiosyncratic: chloramphenicol, anti-inflammatory drugs, antiepileptic agents, gold
Viruses
Epstein-Barr virus (infectious mononucleosis)
Hepatitis
Parvovirus B19
HIV
Immune diseases
Eosinophilic fasciitis
Hypimmunoglobulinemia
Thymoma
Pregnancy
Paroxysmal nocturnal hemoglobinuria
Preleukemia
Idiopathic
Inherited
Fanconi anemia
Dyskeratosis congenita
Shwachman-Diamond syndrome
Reticular dysgenesis
Amegakaryocytic thrombocytopenia
Familial aplastic anemias
Preleukemia, myelodysplasia, monosomy 7
Nonhematologic syndromes (eg, Down, Dubowitz, and Seckel's syndromes)

Modified from Alter and Young [47].

Table 3

Classification of the single cytopenias

Pure red cell aplasia	Neutropenia	Thrombocytopenia
Acquired		
Idiopathic	Idiopathic	Idiopathic
Drugs, toxins	Drugs, toxins	Drugs, toxins
Immune		
Thymoma		
Transient erythroblastopenia of childhood		
Inherited		
Diamond Blackfan anemia	Kostmann syndrome	Amegakaryocytic
	Shwachman-Diamond syndrome	Thrombocytopenia absent radii syndrome
	Cyclic neutropenia	
	Reticular dysgenesis	

Modified from Alter and Young [47].

have equal results, with a slight superiority of BMT demonstrated in younger patients. Second, in patients with SAA whose neutrophil counts are higher than $0.5 \times 10^9/L$, immunomodulation is superior, especially in patients over 40 years of age. This study will have to mature considerably in order to evaluate late morbidity for the two treatment choices.

Recently, Young and Barrett [9••] reviewed the available literature and concluded that young patients with HLA-identical sibling donors should undergo allogeneic BMT, if possible prior to extensive transfusion therapy, using cyclophosphamide and ATG as a preparatory regimen. Those patients without a related donor and older patients should undergo medical management with immunomodulation including ATG, prednisone, cyclosporine, and GM-CSF. For those patients who are refractory to immunomodulation or who relapse after completing therapy, a BMT from an unrelated donor can be attempted with a more intensive preconditioning treatment of cyclophosphamide and ATG with the addition of total body or total lymphoid irradiation. The high incidence of acute and chronic GVHD remains a formidable obstacle to success in these patients.

Fanconi anemia

Fanconi anemia (FA) is a bone marrow failure syndrome characterized by aplastic anemia and congenital abnormalities [10••]. The median age at presentation of a hematologic abnormality is 7 years (range, birth to 31 years) [11•]. The initial finding was thrombocytopenia in 38% of patients and pancytopenia in 53%. The diagnosis of FA is confirmed by demonstrating hypersensitivity to DNA cross-linking agents such as diepoxybutane or mitomycin C resulting in characteristic chromosomal breakage. More recently, an intrinsic cell cycle abnormality, a G2 arrest of stimulated lymphocytes, in response to diepoxybutane, mitomycin C, or nitrogen mustard has been confirmed as a diagnostic test [12,13]. In a double-blind study comparing chromosomal

breakage with cell cycle analysis, concordant results were found in 63 of the 66 patients with FA [14•]. All three patients with discordant results and one with the concordant results had overt leukemia. The optimal diagnosis of possible FA should include both techniques especially for those patients thought to have FA with leukemia or myelodysplasia.

Kohli-Kumar *et al.* [15•] reported on the results of 18 patients with FA who underwent allogeneic BMT from matched sibling donors, all with severe bone marrow aplasia at the time of transplantation, without evidence of a clonal abnormality or malignancy. The median age at BMT was 7.6 years (range, 2.7 to 12.6 years). The usual high-dose BMT preparative regimens lead almost invariably to toxic deaths as a consequence of the underlying defect in DNA repair. Therefore, the preparative regimen used by Kohli-Kumar *et al.* [15•] consisted of low-dose cyclophosphamide, ATG, and thoracoabdominal irradiation with cyclosporine, prednisolone, and ATG as GVHD prophylaxis. There were no toxic deaths; the median time to an absolute neutrophil count higher than $0.5 \times 10^9/L$ was 12 days and to a platelet count higher than $50 \times 10^9/L$ was 22 days. Seventeen patients are alive with a sustained graft at a median follow-up of 27 months (range, 6 to 75 months). Three of the patients have chronic GVHD that responded to steroids and cyclosporine; none of the patients had acute GVHD.

Four FA complementation groups termed A, B, C, and D have been demonstrated by somatic cell hybridization experiments. Strathdee *et al.* [16] cloned the gene defective in FA complementation group C (FACC). A mutation of the FACC gene, denoted IVS4+4A-T, accounts for most cases of FA in Ashkenazi Jewish patients. Further studies of additional Jewish patients with FA revealed 83% to carry this mutant allele [17]. The carrier frequency in the Ashkenazi population is 0.64%. None of 130 non-Ashkenazi patients with FA tested carried this mutation.

Since the identification of the *FACC* gene, the function of its gene product has been studied intensively. Segal *et al.* [18••] have recapitulated the FA phenotype in cell culture using an *FACC* gene antisense oligodeoxynucleotide. Youssoufian [19] surprisingly localized the FACC protein, not to the nucleus but to the cytoplasm, binding to a family of unique cytosolic proteins [20•] that may serve as "cytoplasmic anchors" for FACC. How FACC protects the cell from damage is under investigation.

Fanconi anemia has become a candidate disorder for gene therapy with the cloning of the *FACC* gene. Walsh *et al.* [21••] demonstrated efficient transduction, expression, and phenotypic correction in FA(C) patient-derived lymphoblastoid cell lines using a recombinant adeno-associated virus vector containing the newly cloned *FACC* gene. Limited clinical studies are currently underway.

Dyskeratosis congenita

Dyskeratosis congenita is a rare form of ectodermal dysplasia in which approximately half of the patients develop bone marrow failure. Although it is tempting to postulate a stromal defect in these patients, successful BMT and the results of *in vitro* long-term bone marrow culture studies strongly suggest a stem cell rather than a microenvironmental defect. Nearly 90% of the published cases are inherited as an X-linked recessive disorder, with the remaining 10% being autosomal recessive or autosomal dominant. The classic diagnostic triad of dyskeratosis congenita consists of dystrophic nails, reticulated hyperpigmentation of the skin, and leukoplakia of the mucous membranes. A comprehensive analysis by Drachtman and Alter [22•] of 215 patients gleaned from the literature reveals the mean age of presentation of the characteristic findings to be earliest in the autosomal recessive patients, followed by the X-linked recessive and the autosomal dominant patients. The degree of pigmentation is known to increase with age. These patients have a marked predisposition to malignancy, especially at sites of leukoplakia.

Rarely, the first manifestation of dyskeratosis congenita may be SAA, which occurs at a median age of 12 years and 14 years in the X-linked recessive and autosomal recessive patients, respectively. SAA is extremely rare in the less severely affected autosomal dominant patients. Androgens have been used to treat marrow aplasia in dyskeratosis congenita with varying degrees of success; most often supportive care with blood transfusion is required. BMT has been effective in four of 10 patients studied. Mortality in the remaining six patients was secondary to complications related to BMT. Although this is a small number of patients to analyze, there appears to be an excessive complication rate that may be related to the, as yet undetermined, underlying defect in dyskeratosis congenita. Recent *in vitro* data has suggested that these patients may be responsive to hematopoietic growth fac-

tors. Indeed, GM-CSF administration has been moderately effective in two patients. More recently, Oehler *et al.* [23] reported on one patient in whom G-CSF produced a dose-dependent rise in the neutrophil count to normal. Additional clinical experience is anticipated.

The pathophysiology of dyskeratosis congenita has yet to be elucidated. Dokal and Luzzatto [24], confirming their previous work, studied bone marrow from three patients with dyskeratosis congenita and found the oldest to have unbalanced chromosomal rearrangements in the absence of any inducing agents. This finding correlates with the usual clinical course of dyskeratosis congenita in which malignancies are seen at a later age [22•]. These laboratory studies must be generalized to more patients and confirmed in additional laboratories before dyskeratosis congenita can be categorized as a disorder of chromosomal breakage or instability.

Red cell aplasia

Diamond Blackfan anemia

Diamond Blackfan anemia (DBA) is a rare pure red cell aplasia of childhood characterized by severe anemia, reticulocytopenia, and macrocytosis, generally with a paucity of erythroid precursors found in the bone marrow [25]. Ninety percent of patients are diagnosed during the 1st year of life, 75% by 6 months of age. Approximately 25% of patients have associated congenital anomalies [26]. DBA is frequently distinguished from transient erythroblastopenia of childhood (TEC) only when recovery is observed in those patients with the self-limited disease.

Initially, erythrocyte transfusions are required to stabilize most newly diagnosed patients with DBA. Subsequently, approximately 80% of patients will have a response to prednisone, or another corticosteroid, attaining an adequate hemoglobin. For those patients initially refractory or no longer responsive to steroids, or for those who cannot be tapered to an acceptable every other day schedule, chronic erythrocyte transfusion is the only current treatment alternative. Because this group may represent up to 50% of all patients with DBA [26], other treatment modalities are being investigated. Multiple trials employing interleukin-3 have reported success rates estimated to be between 10% and 20% [27,28]. Ten patients with DBA underwent BMT at a median age of 6 years (range, 1 to 14 years) [29•]. Six of the eight patients who received HLA-identical sibling transplants are currently alive. The two non-sibling donor patients died within 2 weeks of BMT. Currently BMT for selected patients using HLA-identical siblings seems warranted.

The pathogenesis of DBA is gradually being elucidated. The consensus among current investigators is that the majority of cases result from a heterogeneous intrinsic disorder of erythroid proliferation and differentiation [30]. Perdahl *et al.* [31] recently demonstrated accelerated

apoptosis in erythropoietin-deprived erythroid progenitors of patients with DBA, consistent with an intrinsic erythroid progenitor defect. Whether this accelerated apoptosis is the primary defect or, as suspected, the common final pathway of erythroid failure due to a variety of other abnormalities is currently under investigation.

It has recently been realized that the differential diagnosis for pure red cell aplasia in children includes parvovirus B19 infection as well as TEC and DBA. Parvovirus B19 is known to have specific tropism for erythroid progenitor cells, leading to a reticulocytopenia approximately 10 days after infection. A comprehensive review by Heegaard and Hornsleth [32] summarizes the parvovirus-associated diseases, including chronic erythrocyte failure, in otherwise normal patients. The authors point out that parvovirus B19 does not appear to be an important cause of TEC. However, parvovirus infection may mimic DBA in the very young patient and must be ruled out as a cause of pure red cell aplasia before a diagnosis of DBA is made.

Transient erythroblastopenia of childhood

Transient erythroblastopenia of childhood is a self-limited acquired red cell aplasia characteristically observed in previously well children. It presents as a normocytic, normochromic anemia, occasionally severe, with reticulocytopenia. The median age at diagnosis is approximately 2 years. Miller and Berman [33], however, reported six cases of TEC diagnosed in infants between the ages of 3 and 6 months. The article points out the importance of differentiating young patients with TEC from those with DBA.

Cherrick *et al.* [34•] reviewed 50 cases of TEC diagnosed between September 1983 and September 1991. The patients ranged in age from 5 to 44 months, with a mean age of 22.3 months. Nine patients required transfusions. The patients were divided into two groups based on reticulocyte count at presentation. The statistically significant hematologic differences between the two groups at presentation were increased anisocytosis and reticulocyte count ($> 2\%$) in group 2 ($n = 14$), consistent with early recovery, and lower absolute neutrophil and reticulocyte ($< 2\%$) counts in group 1 ($n = 36$). Evaluation for parvovirus was negative in the 15 patients tested. Of greatest interest was the recognition that mild to moderate neutropenia, more pronounced in group 1 patients, but observed in 50% of group 2 patients, is commonly encountered in TEC.

Neutropenia

Kostmann syndrome

Chronic neutropenias are single cytopenias characterized by a decrease in the number of circulating neutrophils. These may be congenital or acquired. Kostmann syndrome is a rare severe congenital neutropenia characterized by an arrest in neutrophil precursor differentiation at

the promyelocyte-myelocyte stage. These patients are usually diagnosed early in the 1st year of life when they present with repeated serious bacterial infections. The risk of infection and its severity correlate with the absolute neutrophil count. Supportive measures with aggressive antibiotic treatment had been the mainstay of therapy, with appropriate patients undergoing BMT.

With the availability of recombinant G-CSF, clinical studies were initiated. To date many patients have benefited from G-CSF with a dose-dependent rise in the absolute neutrophil count and a significant reduction in the morbidity and mortality associated with infections. A comprehensive review by Bonilla *et al.* [35••] examines the consequences of long-term G-CSF use. Forty-four patients with Kostmann syndrome were placed on a phase I-II trial of G-CSF from August 1987 to March 1990. The patients were started on 3 $\mu\text{g/kg/d}$ of G-CSF with 90% demonstrating a complete response (*ie*, median absolute neutrophil count $\geq 1.0 \times 10^9/\text{L}$). Two patients (5%) had a sustained partial response and two patients failed to respond. The final dose of G-CSF ranged from 0.8 to 50 $\mu\text{g/kg/d}$. Three patients had a diminished response in the 2nd year of maintenance treatment. Median platelet counts decreased, whereas median lymphocyte counts were unaffected. Most patients did not have an improvement in what was felt to be the anemia of chronic disease. Clinically, the patients with Kostmann syndrome had significantly less severe infections, *ie*, sepsis, abscesses, and pneumonias requiring intravenous antibiotics, accompanied by a concomitant increase in the incidence of milder infections such as chronic otitis, sinusitis, and upper respiratory illnesses requiring oral antibiotics. Mouth ulcers and gingivitis were also markedly reduced. Adverse events associated with the drug itself included rare local reactions and splenomegaly. Seven patients required discontinuation of the drug. Two patients were able to restart the drug at a lower dose. One patient developed acute monoblastic leukemia 6 months after discontinuing therapy. Three other patients developed myelodysplasia (two with monosomy 7, one transiently); one progressed to acute myeloblastic leukemia. One patient was noted to have marrow fibrosis after 2 years of G-CSF treatment.

Imashuku *et al.* [36] confirmed these findings by reporting four additional cases of myelodysplasia and acute myeloid leukemia in patients treated with G-CSF. Three of these patients had severe aplastic anemia and one had Kostmann syndrome. Two of the three patients with SAA and the patient with Kostmann syndrome developed monosomy 7 and subsequent leukemia. The third patient with SAA developed myelodysplasia without monosomy 7. The interval from diagnosis of the underlying disease to myelodysplasia or leukemia ranged from 2.6 to 3.2 years, and the interval from initiation of G-CSF to myelodysplasia or leukemia ranged from 23 to 31 months. Weinblatt *et al.* [37] also reported on an infant who devel-

oped monosomy 7 after 11 months of G-CSF therapy that progressed to acute nonlymphoblastic leukemia in 7 months.

In addition, Bonilla *et al.* [35••] reported osteoporosis or osteopenia in 15 patients, six having had some radiographic evidence of vertebral changes prior to initiation of G-CSF. A report by Bishop *et al.* [38] quantified bone loss in a 5-year-old child with Kostmann syndrome who presented with vertebral collapse after 2.5 years of G-CSF treatment. Bone mineral content was significantly reduced, and there was evidence of reduced new bone formation and excessive bone resorption. Whether this phenomenon is secondary to the disease itself, and is accelerated by the use of G-CSF, deserves further investigation.

Kostmann syndrome is a single cytopenia with a preleukemic predisposition that may be accelerated by G-CSF treatment or may show increased frequency as patients experience a longer life expectancy. Intensive follow-up is necessary for recognition of myelodysplasia and discontinuation of G-CSF. These data are a sobering reminder that intrinsic progenitor disorders may have a high prevalence of evolution to myelodysplasia or leukemia and that definitive treatment in the form of BMT should be considered for appropriate candidates.

The pathophysiology of Kostmann syndrome is being actively investigated. Mononuclear cells from patients with Kostmann syndrome have been found to produce G-CSF, occasionally at elevated levels, suggesting that the underlying defect may involve cytokine hyporesponsiveness. Indeed, cultured marrow cells have been noted to have a markedly suppressed response to G-CSF. G-CSF receptor studies, however, show normal or increased numbers of receptors with normal affinity for G-CSF. Clinical studies with recombinant G-CSF over the past 6 to 8 years have shown dramatic responses with a significant increase in circulating neutrophils and an improvement in clinical status, whereas GM-CSF has not been effective, suggesting a specific defect in G-CSF signal transduction in these patients. Dong *et al.* [39] reported the identification, in one of six patients studied, of a somatic point mutation in one allele of the G-CSF receptor gene resulting in a cytoplasmic truncation of the receptor and a subsequent defect in signal transduction, leading to defective maturation. Other groups studied 14 patients and documented no receptor mutations [40,41]. These data strongly suggest that mutations in the G-CSF receptor are only a rare cause of Kostmann syndrome.

Congenital cyclic neutropenia

Although not strictly a bone marrow failure syndrome, cyclic neutropenia, or more aptly named cyclic hematopoiesis, is a disorder of bone marrow homeostasis. As such, an understanding of this disorder sheds considerable light on hematopoietic cell regulation. Congenital

cyclic neutropenia is characterized by regular oscillations in neutrophil counts from normal to profound neutropenia, with oscillations in monocyte, eosinophil, platelet, and reticulocyte counts as well. The cycle period is generally 21 days, ranging in some patients from 14 to 36 days. Children present in early childhood with repeated infections, most often with oral ulcers, stomatitis, pharyngitis, or lymphadenopathy. A diagnosis depends on documentation of two cycles of oscillations in the neutrophil count.

Treatment of cyclic neutropenia includes supportive care with antibiotics for infectious episodes and, more recently, use of a hematopoietic growth factor. Neutrophil responses to G-CSF correlate with the resolution of clinical symptoms as nadirs generally rose to safe levels in spite of continued oscillations. Bonilla *et al.* [35••] included 10 patients with cyclic neutropenia in her previously mentioned evaluation of G-CSF therapy for neutropenic patients. All 10 previously untreated patients had a complete response to G-CSF on a daily dose of 3 to 5 $\mu\text{g/kg/d}$ and could be tapered successfully to alternate day therapy. None of the patients with cyclic neutropenia were noted to develop myelodysplasia or leukemia.

Of great interest, Wright *et al.* [42••] compared the effects of GM-CSF with those of G-CSF in a crossover study involving three patients with childhood-onset cyclic neutropenia. The GM-CSF treatment significantly dampened the cyclic oscillations in neutrophil as well as monocyte, platelet, and reticulocyte counts. However, the magnitude of the GM-CSF stimulated increase in neutrophil counts was only 1.6- to 3.9-fold. GM-CSF did, however, induce marked eosinophilia in these patients. In contrast, G-CSF elevated peak neutrophil counts by more than 20-fold. Although the cycling of neutrophils as well as the other blood elements was amplified, the nadirs were sufficiently elevated to eliminate or significantly reduce clinical symptoms. Eosinophilia was not noted with G-CSF. Thus despite the differences in the neutrophil counts, similar clinical improvement was noted with each growth factor. However, the authors warn of the potential adverse effects of GM-CSF-induced hypereosinophilia. That each growth factor has distinct effects on hematopoiesis in this disorder supports the notion that the defect in cyclic neutropenia resides in progenitors prior to and including the colony-forming unit granulocyte macrophage. Schmitz *et al.* [43••] support this notion and propose a "two feedback" model for the regulation of granulopoiesis. In this model, GM-CSF regulates the mitotic activity of the colony-forming unit granulocyte macrophage compartment and is controlled by the total "granulopoietic bone marrow cell count," whereas G-CSF is controlled by a much tighter feedback loop influenced only by the size of the circulating neutrophil compartment and influencing the mitotic activity of only the colony-forming unit granulocyte and the bone marrow neutrophil precursors.

Thrombocytopenia with absent radii

Thrombocytopenia absent radii syndrome is generally diagnosed at birth because of the characteristic intercalary orthopedic abnormality, absence of bilateral radii with the presence of thumbs, associated with thrombocytopenia. Prenatal diagnosis has been made by imaging techniques detecting skeletal abnormalities. Weinblatt *et al.* [44] reported on a fetus with bilateral absent radii who was followed up by serial ultrasound. A blood count done at 37 weeks' gestation revealed thrombocytopenia. The infant received an intrauterine platelet transfusion and was delivered without complications, thus decreasing the risk of perinatal bleeding.

The thrombocytopenia of thrombocytopenia absent radii syndrome generally resolves by 1 year of age, without further major hematologic abnormalities. This syndrome is not reported to progress to aplastic anemia or leukemia. However, Symonds *et al.* [45] reported on a 70-year-old woman with bilateral absent radii who developed marked pancytopenia. This patient also developed three separate primary cancers. *In vitro* testing of the patient's lymphoblastoid cell line revealed abnormal radiosensitivity, suggesting a similarity to other abnormal DNA repair syndromes.

Conclusions

This year has been marked by extraordinary progress. Over the next few years novel treatments for bone marrow failure should develop from new knowledge. If permitted, innovative clinicians will translate these strategies into decreased morbidity and increased survival for these complex patients.

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- Of special interest
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Medical treatment of children with SAA treated with ATG, prednisone, cyclosporine, and GM-CSF is beneficial. This regimen results in a decrease in initial morbidity, which the authors postulate is due to the decrease in associated infections secondary to an early increase in the neutrophil count.

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